Use of Milt From On-Year Males in Transplants to Establish Off-Year Pink Salmon *(Oncorhynchus gorbuscha)* Runs

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Les numéros 1-456 de cette série ont été publiés à titre de Rapports techniques de l'Office des recherches sur les pêcheries du Canada. Les numéros 457-714, à titre de Rapports techniques de la Direction générale de la recherche et du développement, Service des pêches et de la mer, ministère de l'Environnement. Les numéros 715-924 ont été publiés à titre de Rapports techniques du Service des pêches et de la mer, Ministère des Pêches et de l'Environnement. Le nom de la série a été modifié à partir du numéro 925.

La page couverture porte le nom de l'établissement auteur où l'on peut se procurer les rapports sous couverture cartonnée.

ERRATA SHEET

p. 10, line 31. The expression $K_D = \frac{\sqrt[10]{W \text{ in mg}}}{L \text{ in mm}}$

is incorrect and should be replaced by

$$K_D = \frac{10\sqrt[3]{W \text{ in mg}}}{L \text{ in mm}}$$

p. 28, line 10. The expression ($\chi^2 = 0.038$, 1 d.f.; p. .05)

is incorrect and should be replaced by

($\chi^2 = 4.29$, 1 d.f.; p. 0.04)

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USE OF MILT FROM ON-YEAR MALES IN TRANSPLANTS TO ESTABLISH OFF-YEAR PINK SALMON (*Oncorhynchus gorbuscha*) RUNS

by

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ABSTRACT

Withler, F. C., and R. B. Morley. 1982. Use of milt from on-year males in transplants to establish off-year pink salmon (Oncorhynchus gorbuscha) runs. Can. Tech. Rep. Fish. Aquat. Sci. No. 1139: iv + 49 p.

An experiment was carried out on Northeastern Vancouver Island, British Columbia, to discover whether or not the return of adults from pink salmon ova from a donor stock, planted in an on-off stream in the off year, could be improved by introducing genes from the receiving stream's on-year run. Ova from the Glendale River, B.C. were fertilized by cryogenically-stored sperm from the Bear River on-year run and by sperm from mature one-year-old males derived from the same run. The resulting (treatment) fry were marked by finclipping and released in 1976 in company with marked (control) fry from Glendale ova fertilized by sperm from the Glendale run. The pure Glendale type fry had hatched and emerged earlier, at an earlier stage of development, than had the hybrid type.

No marked adult pink salmon of either type returned to the Bear River in 1977, although marked adults were recovered in the Juan de Fuca and Johnstone straits commercial fisheries of southern B.C. Some of the marked adult pinks appeared to have congregated near the Bear River estuary. Significantly more of the pure Glendale type than hybrid type adults were found among the commercial catches, and the Glendale type females were longer and heavier than the hybrid females.

Key words: Pink salmon, transplants, on-offness, genetic differences, refugia, crygogenic sperm storage, gonadotropin, hatching, emergence, finclipping, mark recovery

RÉSUMÉ

Withler, F. C., and R. B. Morley. 1982. Use of milt from on-year males in transplants to establish off-year pink salmon (Oncorhynchus gorbuscha) runs. Can. Tech. Rep. Fish. Aquat. Sci. No. 1139: iv + 49 p.

Une expérience a été effectuée au nord-est de l'île Vancouver, en Colombie-Britannique, afin de découvrir s'il était possible, grâce à l'introduction de gènes venant de saumons remontant à leur cours d'eau natal lors d'une année paire, d'améliorer le retour des saumons roses adultes issus d'oeufs provenant d'un stock donneur et ensemencés au cours d'une année impaire dans un cours d'eau où les remontes varient entre années paires et impaires. Les oeufs provenant de la rivière Glendale (C.-B.) ont été fécondés par du sperme conservé par cryogénisation provenant de saumons remontant la rivière Bear au cours d'une année paire et par du sperme de mâles adultes âgés d'un an provenant de la même montaison. Les alevins (expérimentaux) qui en

ont résulté ont été marqués par rognage des nageoires et ensemencés dans 1976 en compagnie d'alevins marqués témoins issus d'oeufs de la rivière Glendale fécondés par du sperme provenant de saumons remontant la rivière Glendale. Les alevins du type pur Glendale ont éclos plus tôt et sont apparus à un stade moins avancé de développement que ne l'ont fait les alevins du type hybride.

En 1977, il n'y a eu aucun retour de saumons roses marqués des deux types à la rivière Bear, bien que des adultes marqués aient été recapturés par les pêcheurs commerciaux dans les détroits de Johnstone et de Juan de Fuca au sud de la C.-B. Certain des saumons roses adultes marqués se sont, semble-t-il, rassemblés près de l'estuaire de la rivière Bear. De façon significative, on a trouvé un plus grand nombre d'adultes du type pur Glendale que du type hybride parmi les prises commerciales et les femelles de type Glendale avaient une plus grande taille et pesaient davantage que les femelles hybrides.

Mots-clés: saumon rose, ensemencements, montaison aux deux ans, différences génétiques, refuges, conservation du sperme par cryogénisation, gonadotropine, éclosion, émergence, rognage des nageoires, récupération des poissons marqués

INTRODUCTION

The almost invariable two-year life cycle of pink salmon (Oncorhynchus gorbuscha) effectively divides the species into two genetic lines maturing in alternate years. Throughout their geographic range there are often marked differences in abundance between lines, the extreme situation being that of complete dominance where one or other line is virtually absent (Ricker 1962). The magnitudes of the "on-year" runs in many such streams (e.g. the Fraser River in British Columbia) have encouraged attempts to establish missing lines, usually by transfer of fertilized eggs from donor streams.

Neave (1965), in a review of pink salmon transplants up to that time, concluded that there had been no successful attempt to establish a substantial, self-sustaining run in an off-year within the natural range of the species. Since then a transfer of mature adults to Sashin Creek, Alaska (McNeil et al. 1969) may have resulted in a continuing off-year population. However, there was a possibility of persistence of a small residual population in the off year at the time of the transplant and the situation regarding completeness of dominance prior to the introduction was unclear. With this possible exception, all attempts to establish missing lines of pink salmon in waters to which they are indigenous have either failed to produce returning adults or the resulting runs have declined rapidly to extinction.

Neave (1965) discusses reasons for the repeated failures, among them the possibility of genetic barriers to transplantation. Evidence now exists that salmon stocks possess hereditary characteristics suited to the conditions of their particular spawning and rearing areas (Brannon 1967; Ricker 1972). Ricker's (1972) observation that ability to "home" is also partly under genetic control has now been demonstrated conclusively for pink salmon in an experiment by Bams (1976) in which adults resulting from transplanted eggs fertilized by males of the recipient stream homed to the recipient stream better than did those produced from transplanted eggs fertilized by donor stream males. If generally applicable, these results bear directly on the possibility of successful pink salmon transplants.

The experiment reported here was designed to test the effectiveness of Bams' technique of "donor-recipient hybridization" as an aid in establishing missing lines of pink salmon. The test differs from Bams' in one important aspect. While he was able to use recipient stream males from a small residual off-year population to fertilize donor eggs, the recipient stream in our case exhibits virtually complete dominance of one line. Therefore, the sperm necessary to fertilize the donor eggs were not directly available in the off-year. Two techniques were developed to overcome this problem:

(1) Cryogenic preservation of sperm, allowing sperm from on-year males to be stored and used the following year,

(2) Acceleration of growth and maturation of on-year juveniles to produce large, sexually mature 1-year-old males.

Given these techniques, it became feasible to test the effect of transfer of the male component of the successful on-year gene pool into the ova of a donor stock introduced in the off-year cycle.

MATERIALS AND METHODS

CHOICE OF STREAMS

Bear River (gazetted name Amor de Cosmos Creek) was selected as the recipient stream for the transplant. This small river, situated on northeastern Vancouver Island and draining into Johnstone Strait (Fig. 1), had strong even-year pink escapements in the order of 35,000 to 130,000 up to 1974. The odd-year run is virtually non-existent, pinks (a maximum of 25) having been observed on only 3 occasions from 1947 to 1975 (Marshall et al. 1977). This on-off characteristic is shared by some other streams in the vicinity.

The donor stream chosen was the Glendale River, draining into Knight Inlet on the mainland side of Johnstone Strait, 33 km directly north of Bear River but approximately 110 km distant by the shortest water route. It supports runs of pink salmon in both odd and even years. Selection of this stream was based on several criteria, including recent strength of runs, coincidence of timing with the on-year Bear River run, practicality of collecting and transporting the required gametes, and compatibility with respect to transmittable diseases as determined by surveys carried out in 1974.

In the spring and summer of 1975 a hatchery was constructed at Bear River and an old weir and holding ponds at Glendale River were renovated in preparation for the test that fall.

TEST DESIGN

The test was designed primarily to allow comparison of two introduced groups - a "pure" donor stock and a "hybrid" stock created by fertilizing donor eggs with sperm from recipient stream males. To compare the effectiveness of the 2 methods of obtaining Bear River sperm (cryogenic storage *vis à vis* accelerated males), sperm from both sources were used. In addition, a substantial number of eggs of Glendale ♀ x Glendale ♂ origin were incubated. These are referred to hereafter as "non-test". Most of the fry from non-test eggs were released at the same time as the test groups to act as buffers against predation on the test groups after release. The various crosses are shown below, together with descriptive labels used for later reference:

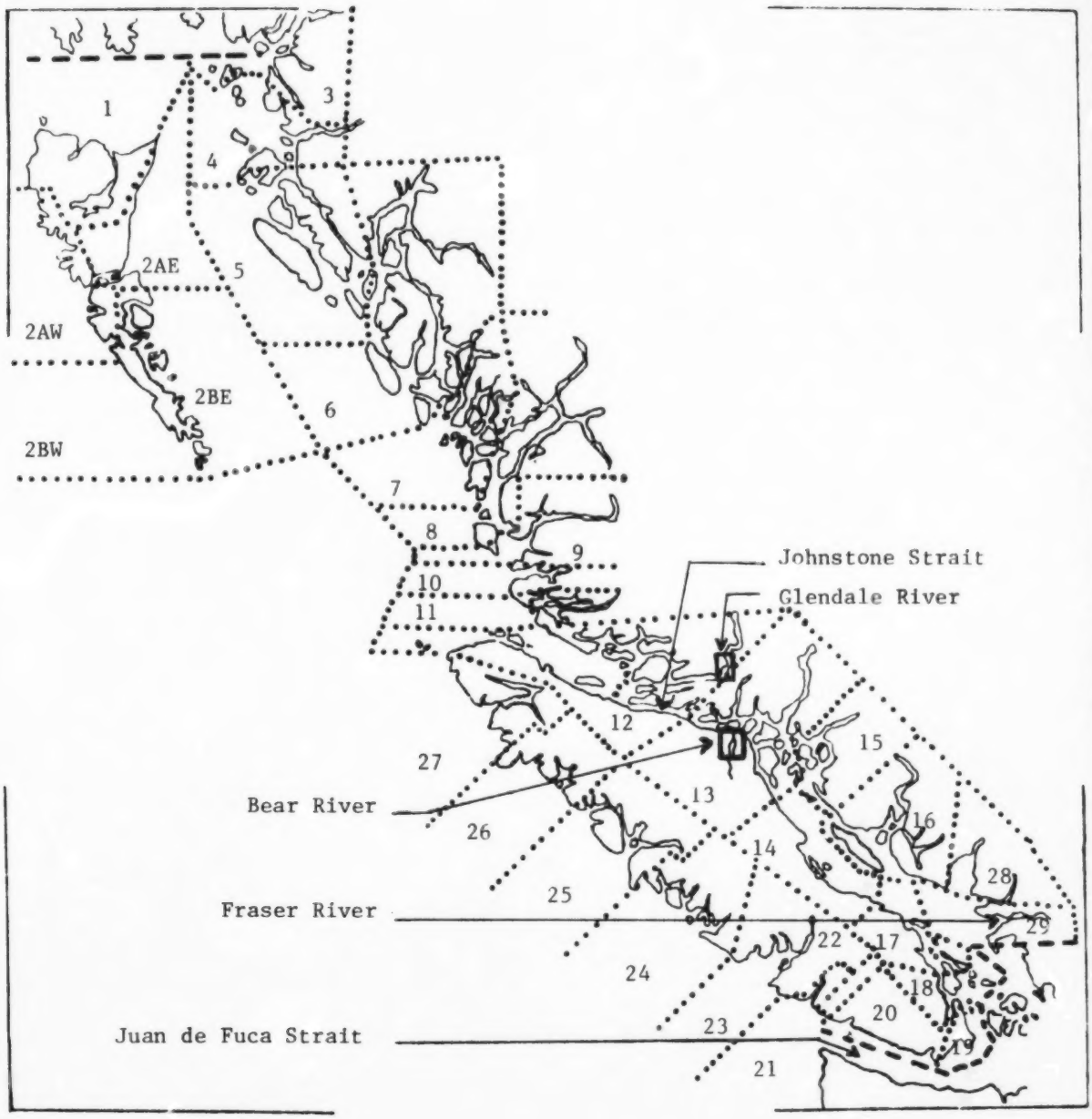

Fig. 1. British Columbia Statistical Areas used for designating origins of salmon catches. Other sites referred to in the text are shown.

	Cross	Source of Sperm	Descriptive label
Test groups	Glendale ♀ x Glendale ♂	1975 Glendale River males.	Control group, or "C".
	Glendale ♀ x Bear ♂	1974 Bear River males, sperm frozen for 1 year.	Frozen sperm group, or "FS"
	Glendale ♀ x Bear ♂	1-year-old males, derived from the 1974 Bear River run.	Accelerated male group, or "AM".
Non-test	Glendale ♀ x Glendale ♂	1975 Glendale River males.	Non-test group, or "NT".

Ova and some milt (for the Glendale ♀ x Glendale ♂ crosses) were collected at Glendale River and transported to the Bear River hatchery. Here the ova were fertilized by sperm from one of the three sources and incubated. A portion of the fry from each test group was distinctively marked at emergence by fin-clipping and released into Bear River (details of collection, fertilization, incubation, and marking follow). As much as possible the three groups received equivalent treatment.

Test criteria included fertilization rates; time of hatch and emergence; survival to fry stage; length, weight and stage of development at emergence; fry to adult survival; and ultimately, rate of return to Bear River. Rather than have returning adults of unknown origin, the only unmarked fish released were those resulting from Glendale ♀ x Glendale ♂ crosses. These included Control group fish surplus to those needed for marking plus non-test (NT) fry.

BEAR RIVER SPERM

Milt for the frozen sperm (FS) group was collected from natural spawners of the 1974 Bear River run. Freezing methods used were derived from techniques developed at Oregon State University (Horton et al. 1967). The method used in this experiment is outlined in Appendix A. Ninety-five males captured from the central two-thirds of the run contributed to the sperm bank. Milt from 3 to 6 individuals was pooled prior to dilution and freezing. It was frozen during the period October 17-30, 1974 and stored in liquid nitrogen at the Pacific Biological Station until the following year.

Milt for the accelerated male (AM) group was stripped from 1-yr-old males at the time of fertilization. These fish were the progeny of 22 females and 18 males of the 1974 Bear River run captured October 20, 1974. Eggs and sperm were transported to Nanaimo where the gametes of each type were pooled prior to fertilization to ensure a good mix of parental origins. Incubation and early rearing were carried out at the Pacific Biological Station. Fry were then transferred to the West Vancouver Laboratory for use by Colin N. MacKinnon in a study of regulation of reproductive development in pink salmon. Although various techniques of advancing maturation in both

sexes were investigated, only males were matured successfully at age one year. Males of one of the treatment groups were used in this experiment. Treatment of these fish had consisted of injecting salmon gonadotropin coupled with incubation and rearing at constant high temperature (≈ 12°C) to promote rapid growth. Methods are outlined in MacKinnon (1976). During the egg take in October 1975, the fish were transferred to fresh water and transported to Bear River. Average weight of these fish was 120.5 g.

Of the 187 males selected for the test, 153 produced milt and 34 did not. The 153 males produced a total of 610 mL of milt, or about 4 mL each. This volume represents about 15% of that produced by a normal male pink at a single stripping (Morley and Withler 1969).

SPAWN-TAKING, FERTILIZATION AND INCUBATION

Collection and transport of donor eggs and sperm

Spawners were collected mainly from the latter part of the Glendale run, beginning in late September. The first spawn-take was on October 6, a date which agreed well with the start of heavy spawning of Bear River fish in on years. Milt and unfertilized eggs were transported by float plane to Bear River where fertilizations were made the same day. Spawn-takes continued at 1 to 2 day intervals until October 18 (10 in total) at which time the Glendale run had completed spawning. About every fifth male and every tenth female taken for spawning were measured.

Milt was held in 250 mL screw-cap amber-glass jars, each containing 90 mL of milt (pooled production of an average of 5.4 males). Eggs were held in 80 fl. oz. (2366 mL) plastic tubs with snap-top lids, each containing 64 fl. oz. (1893 mL) of eggs (pooled production of an average of 7.4 females). For transport, these gamete containers were packed in styrofoam chests, 6 tubs of eggs per chest with up to 4 jars of milt per chest fitted between the egg containers. A small amount of chipped ice was placed between the containers at the bottom of the chest. A sheet of perforated hard-board was then laid on top of the egg-containers and a further 2-3 L of ice in a plastic bag placed on this before sealing the chest. Average temperature of eggs on arrival at the hatchery was 9.4°C (range 6.3-10.8). Milt temperatures were slightly lower (mean 8.5°C, range 4.8-9.7). These temperatures indicate slight cooling (approx. 1-2°C) during transport.

Fertilization procedures

As much as possible, that portion of each day's egg-take used for the three test groups (Table 1) was distributed in the approximate ratio 1C:2FS:1AM (this ratio allowed for expected lower fertilization rates by frozen sperm). An exception were the October 18 fertilizations when no AM group eggs were fertilized. Allocation of ova to the treatment groups was non-selective. Fertilizations were carried out concurrently, although FS fertilizations were usually completed last because they took longer. Trays of eggs containing the various groups were placed randomly within daily

consecutive blocks in Heath vertical stack incubators. Fertilization and washing were carried out in 80 fl. oz. (2366 mL) plastic tubs. Procedures for each group were as follows:

Control group

To 24 fl. oz. (710 mL) of Glendale ova were added 17 mL of Glendale milt, usually from a single jar. Milt and ova were mixed by hand for approximately 1 min. The tub was then filled with water, the contents mixed briefly, and the eggs washed thoroughly using several exchanges of water. Two such 24 oz. portions (approx. 7,750 eggs) were accumulated in a Heath tray of running water and placed immediately in the incubator stack.

Accelerated male group

On each day of fertilization sufficient males were stripped live to produce the required volume of milt. Each male was stripped only once. Milt from individuals was pooled before use, approximately 17 to 40 fish being stripped each day. Fertilization procedure was the same as for the control group except that only 10.5 mL of milt was applied to each 24 oz. portion of ova.

Frozen sperm group

Ova were fertilized in 8 oz. (237 mL) portions using 4 or 5 packages of sperm mixture. Each 2 mL package contained 0.2 mL of diluted milt (see Appendix A). Each portion of milt was stirred into the ova immediately after thawing in a 45°C water bath. A minimum of 1 min after application of the last portion, the tub was filled with water and the eggs stirred and washed as for other groups. Six such 8 oz. portions were accumulated in a Heath tray before being placed in the stacks.

Non-test group

Ova from a shipping container were divided into two 32 oz. (946 mL) portions. Each was then fertilized using 22 mL of Glendale milt in a similar fashion to that for the control group. Two portions were combined in a tray (approx. 10,600 eggs).

Genetic variability

It was impractical to pool all gametes prior to fertilization to ensure completely random fertilization or to completely segregate gametes of individual fish or groups of fish and perform all crosses using a common mating pattern. The genetic "mix" attained in each cross must therefore be assumed to be random and of sufficiently broad range to preclude possible effects of differences in mating patterns between groups. The approximate mating patterns are as follows:

Control group

Pooled milt from 5-6 males was used to fertilize the ova of approximately 15 females.

Table 1. Dates of spawn-taking, numbers of Glendale spawners stripped, and distribution of green and eyed eggs among test and non-test groups. Unbracketed numbers are green eggs placed in incubators after different fertilization treatments; bracketed numbers are numbers of eggs of each test group planted in gravel boxes at the eyed stage. All egg numbers rounded to the nearest 100.

Spawning date	Fish spawned		Control (C)		Frozen sperm (FS)		Accelerated male (AM)		Non-test[a] (NT)	Total
	Females	Males								
Oct. 6, 1975	156	49	39,300	(23,000)	53,900	(24,900)	30,000	(15,100)	111,200	234,400
Oct. 8, 1975	316	60	45,300	(21,400)	58,500	(19,000)	37,800	(28,900)	293,600	435,200
Oct. 9, 1975	222	49	39,900	(29,600)	60,200	(23,400)	38,000	(28,400)	160,800	298,900
Oct. 10, 1975	224	60	38,000	(28,600)	61,000	(27,100)	36,300	(27,800)	164,300	299,600
Oct. 12, 1975	226	75	-	-	-	-	-	-	310,600	310,600
Oct. 14, 1975	133	16	45,300	(34,100)	82,200	(31,100)	40,900	(31,400)	10,900	179,300
Oct. 15, 1975	216	70	38,500	(35,300)	69,100	(38,200)	48,100	(39,700)	143,500	299,200
Oct. 16, 1975	223	82	36,300	(35,000)	82,000	(44,800)	59,900	(43,400)	112,500	290,700
Oct. 17, 1975	213	91	50,900	(38,800)	92,000	(41,600)	76,700	(58,800)	70,900	290,500
Oct. 18, 1975	59	38	31,600	(27,100)	42,200	(21,700)	-	-	19,800	93,600
Total	1,977	590	365,100	(272,900)	601,100	(271,800)	367,700	(273,500)	1,398,100	2,732,000

[a]Most non-test eggs were planted as green eggs in outdoor gravel boxes; several thousand were used in other tests (e.g. incubator tray substrates).

Accelerated male group

The pooled milt from each day's group of males (17 to 40) was used to fertilize the ova of all females contributing to the cross that day (19 to 55).

Frozen sperm group

Very approximately, the pooled milt of a group of about 4 males was used to fertilize the eggs of 20-30 females.

Non-test group

Pooled milt of 5-6 males was used to fertilize the eggs of approximately 15 females.

Incubation

Eggs remained in the Heath Incubators until the eyed stage. Water flow through each stack of 16 trays was maintained at 12 Lpm (no eggs were placed in the top trays). One-hour malachite green treatments (2.2 ppm) were given twice weekly by introducing the chemical into the water supply at the head tank. When fully eyed (300°C days) the eggs were shocked with a jet of water and the dead removed and counted. Volumetric measurements were used to determine the numbers of live eggs. Accuracy of these measurements was determined by manual counts of a series of measured volumes of eggs (Appendix B). On completion of picking, equal numbers from each of the three test groups were placed in indoor incubation gravel boxes, 2 boxes per group. Allocation of treatments to boxes was randomized. Malachite green treatments were discontinued at this point.

Boxes were 1.2 × 1.2 × 2.4 m long (4 × 4 × 8 ft) with an upwelling water supply. They are described in detail by Bams and Crabtree (1976). Depth of the initial gravel layer was 6 in (15 cm) rather than the 4 in (10 cm) used by Bams. Incubation gravel was obtained commercially as 1 1/2 in (3.75 cm) minus size crushed drain rock.[1] Flow to each box of about 136,000 eggs was maintained at 100 Lpm.

All live eggs of the FS group were utilized. Eggs for the AM and C groups were selected so that the various spawn-takes for these groups were represented in proportions similar to those for the FS group. Similar equivalency was maintained for the replicate pairs of planted incubation boxes. AM eggs surplus to those required for planting in the boxes were retained in Heath trays and destroyed after hatching. Surplus C eggs were also hatched in trays and the fry either used for practice marking or released unmarked as buffers.

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[1] Measured range 1/4 to 1 1/2 in (0.6 to 3.8 cm), 80% being 3/4 in (1.9 cm) or larger.

After planting of the test groups, about 60% of the non-test eggs were planted in two outdoor gravel boxes at densities of about 250,000 and 500,000 to obtain information on loading capacity as well as to produce buffer fry. Of the remaining 40% of the non-test eggs, about 120,000 were used in Astro-turf substrate tests and 390,000 were incubated in the Heath stacks. Over 80% were released as unmarked buffer fry. A summary of spawn-taking and distribution of eggs in the incubator stacks is given in Table 1.

HATCHERY DETAILS

Water for the hatchery was fed by gravity to a sump from buried intakes in Bear River and a small tributary, Cold Creek. From the sump, water was pumped through sand filters[2] to a head tank, then distributed to the incubator stacks and upwelling incubation boxes. Cold Creek water was usually several degrees cooler than that from Bear River and regulation of flows to the common sump allowed some degree of temperature control. Cold Creek water was blended with Bear River water mainly prior to planting eggs in the incubation boxes and throughout emergence, and also during the month preceding emergence in order to slow development of the alevins.

Temperature of hatchery water was monitored continuously on a Taylor recording thermograph. Temperatures of Bear River and Cold Creek water were taken daily at 0800 and 1600 hr. Daily mean hatchery water temperature was calculated by averaging temperatures recorded at 0600, 1200, 1800, and 2400 hr. Time-temperature units were calculated as "Celsius degree days" (C° days) of incubation.

HATCHING OBSERVATIONS

At the eyed stage, 2 samples of 100 eggs from each test group were taken from the incubation trays. Fertilization date for one set of these samples was October 14, for the other October 10. Eggs were selected from single trayloads and the samples randomly distributed in a partitioned Heath tray. The eggs were transported to Nanaimo where daily observations were made of mortality, numbers hatched and deformities. Alevins were removed daily and the fresh nose-fork length (nearest 0.5 mm) of every third fish recorded. In addition, single samples of 100 eggs from the C and AM groups, fertilization date October 6, were observed at Bear River to predict hatching time of the eggs fertilized earliest. No length measurements were taken from these samples.

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[2]Swimquip Commercial Hi-rate Filter Systems. Swimquip of Canada Ltd., 74 Gervais Drive, Don Mills, Ontario.

EMERGENCE

Fry emerged voluntarily from the boxes during darkness and were trapped in buckets. Emergence from the 6 indoor test group boxes was easily regulated by turning the overhead lights on or off and this procedure was used to slow emergence to allow marking of adequate numbers of fry each day. Emergence patterns observed are therefore somewhat artificial. Each morning the emerged fry from each test box were sampled by successively splitting the total emergence down to the approximate sample size of 55.[3] Samples were preserved in 10% formalin for later measurements of length and weight. Fry were then held in buckets or tanks for marking and/or counting. Marked fish were counted by each marker as the marks were applied. At the end of a day's marking, all unmarked test fish were anesthetized and counted. Only 50% of the fry from each box were marked. Excess FS and AM fry were destroyed, excess C fry were released as buffers. All test fish to be released were retained and liberated together at or shortly after nightfall on the day of emergence. On only 3 occasions were small numbers of fry retained for marking the following day.

Emergence from the two outside non-test boxes was controlled to a lesser extent by use of outdoor lights to try to match emergence of these (buffer) fry with that of test fish. These fry entered the river directly, except for a small fixed portion which was trapped as they emerged. This portion was counted to estimate total emergence and released that evening. Fry incubated in the stacks were released periodically along with test fry to even out the bufferring effect. These fry were often well beyond optimum stage for release.

Processing of samples

Fifty fry from each daily sample of 55 were processed after approximately 3 mo preservation. Fork length (to the nearest 0.2 mm) and total wet weight (to the nearest mg) were recorded and individual K_D - values calculated from the formula:

$$K_D = \frac{\sqrt[3]{W \text{ in mg}}}{L \text{ in mm}}$$

This value is used to express stage of development (Bams 1970).

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[3] Splitting was done by pouring fish (in water) into an inclined chute. A divider located at the outlet divided the sample into two approximately equal portions.

MARKING

Marks used

Fry were marked with double fin-clips as follows:

Control group:	adipose-left ventral (AdLV)
Frozen sperm group:	adipose-right ventral (AdRV)
Accelerated male group:	left ventral-right ventral (BV)

Double marks were applied because naturally missing fins occur relatively frequently in wild populations whereas the expected coincidence of two independently occurring natural missing fins is so low as to be negligible (Bams 1972). The assumption of independence of the two natural marks should be noted: it was realized this assumption would probably hold less true for the BV mark which involves immediately adjacent fins. It was also assumed that the symmetrical marks (AdLV, AdRV) would affect survival equally. The comparability of the BV mark to the other two in this regard is questionable.

Marking procedure

Marking closely followed procedures described in detail by Coburn and McDonald (1972). Fish were anaesthetized using 2-phenoxyethanol at a concentration of 1:2642. Temperature of the anaesthetic was maintained to correspond closely to that of the river (4-9°C). A crew of 10 fry markers clipped fins using cornea snips. Marked fry were accumulated in recovery tanks located before each marker, each marker maintaining her own count. Fish remained in these tanks until that night when they were released by removing the standpipes, allowing them to pass into a common drain emptying directly into the river.

Mark evaluation

Fin-clipped fry were sampled for mark quality throughout. Daily samples of 10 fry for each type marked were taken from each marker; occasionally up to 3 samples of a particular mark were taken from a particular marker. Each sample was examined under a microscope and the proportion of invalid marks determined. These included:

- marks in which at least some portion of either of the two fins involved was not completely removed;
- marks in which some portion of an extra fin was removed (e.g. Ad-RV-LV);
- marks in which the wrong fins were removed, placing them in the wrong group.

The proportion of invalid marks observed in each marker's daily sample(s) of a particular mark was calculated and her daily production for that mark reduced accordingly.

Valid marks in a sample were scored as in Coburn and McDonald (1972) on the basis of length of each clipped fin remaining as follows:

3 points:	1/20 or less remaining	
2 points:	1/20 to 1/10	"
1 point:	1/10 to1/5	"
0 point:	> 1/5	"

For a sample of 10 valid marks a perfect score would be 10 × 3 points (first fin) plus 10 × 3 points (second fin) = 60 points. Scores based on a sample of other than 10 fish were adjusted to give a score out of 60.

To compare overall quality of the three marks, seasonal average scores for each marker for each of the 3 marks were first calculated. Combined seasonal averages for each type of mark were then calculated by weighting each marker's seasonal average score for a mark by his total seasonal production for that mark.

No adjustment to mark totals were made on the basis of mark quality; only totally invalid marks (as previously described) were discounted.

ACCURACY OF ESTIMATES TO FRY STAGE

Except for the manual counts of all emergent test fry, numbers of eggs and fry given are based on volumetric estimates made at the eyed stage. Hence, precision of estimates of numbers incubated, numbers of fry emerged and released (other than test fry), and survival are determined by the accuracy of the method (Appendix B). Emergence from the two non-test gravel boxes was estimated by counting a small portion of the total daily emergence captured when they dropped through a small slot in the exit chute. Periodic checks were made to determine the number captured as a proportion of the total emerging and an average proportion calculated.

ADULT RECOVERIES

A mark recovery program was conducted in the summer and fall of 1977 in 3 areas.

Commercial fishery

Commercial catches were examined at processing plants operated by B.C. Packers Ltd. (Steveston, B.C.) and Canadian Fishing Co. Ltd. (Vancouver, B.C.). Special effort was made to sample catches from the Johnstone Strait fishery with occasional checks on fish from other areas. When possible the precise "pick-up points" for each packer load were obtained. Pinks were examined for any missing or incomplete adipose or ventral fins. Sketches of such fins were made for later evaluation. Fork and hypural lengths, weight,

and sex were recorded. Emphasis was placed on recovery of the comparable AdRV and AdLV marks, especially when large numbers were being examined. At such times pinks on the conveyor lines were scanned primarily for missing adipose fins; any with this easily detected mark were then set aside and the ventral fins examined thoroughly later. Each observer maintained a count of the actual number examined by him.

Stream surveys

Glendale River was surveyed by foot on several occasions to examine carcasses of spawned fish. Several small streams in the vicinity of Bear River also were surveyed but to a lesser extent.

Bear River

A fish counting fence was operated near the mouth of Bear River during most of the normal escapement period for on-year runs. Foot surveys of the river and estuary were made subsequent to the fence washing out late in the season. As well, some skin-diving surveys were made in the estuary.

RESULTS

TIMES TO HATCHING

Differences among the 3 types of eggs in time to hatching related to source of sperm used for fertilization (C, FS, AM) were examined using the median numbers of thermal units (C° days) each group experienced up to hatching. (See MATERIALS AND METHODS: HATCHING OBSERVATIONS). The pattern of hatching is illustrated in Fig. 2.

Non-normality (platykurtosis) among the C° day distributions precluded analysis by parametric techniques. However, the generally close agreement between C° day means and medians meant that medians could be used as reliable measures of central tendency, so a non-parametric median test (Seigel 1956) was employed. The means, medians, and 95% confidence limits on the medians were as follows:

October 10 spawning:

C type: $\bar{x}$ 521.5, med. 521.3 (520.4, 522.5)
FS " : $\bar{x}$ 526.8, med. 524.6 (523.9, 525.7)
AM " : $\bar{x}$ 529.5, med. 529.3 (527.3, 531.2)

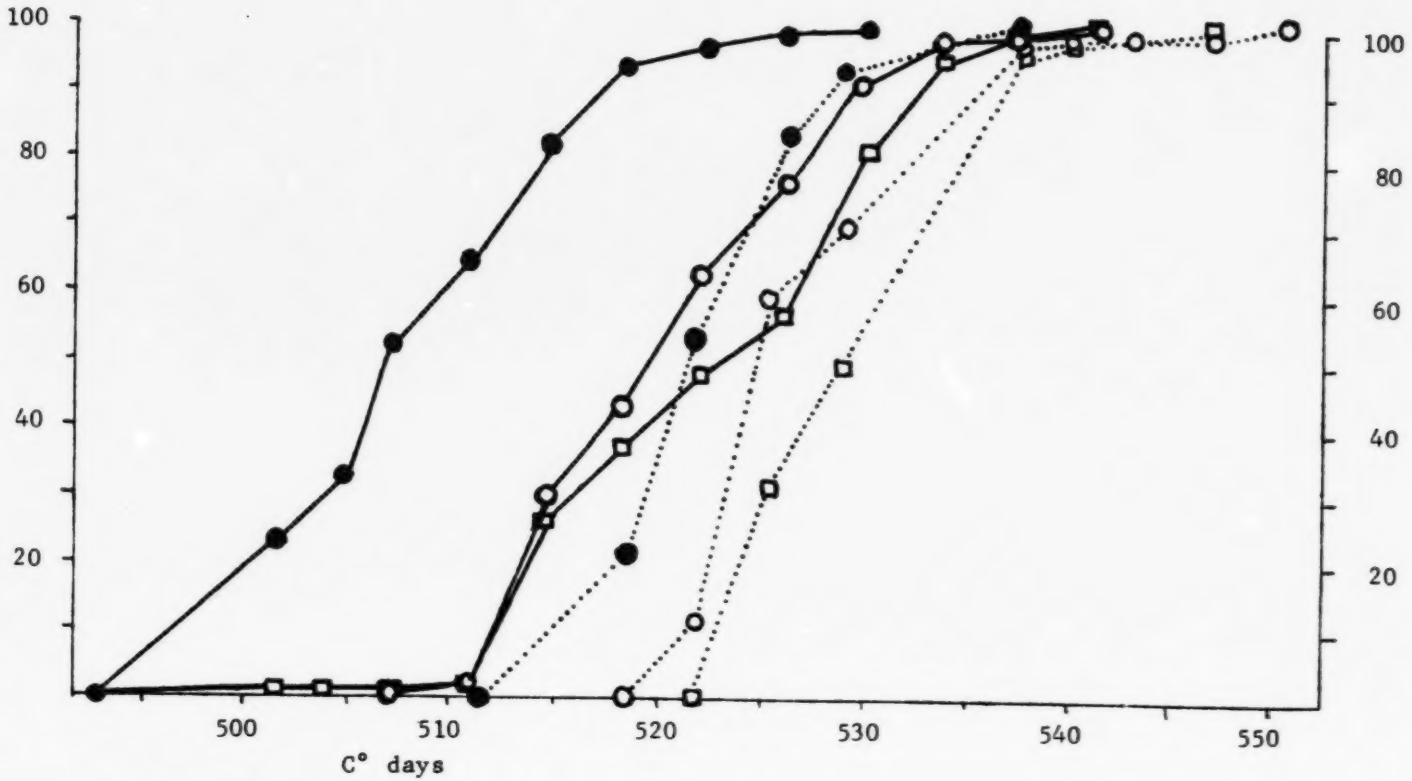

Fig. 2. Cumulative percent hatch of 3 types of pink salmon ova in relation to C° days: closed circles C type, open circles FS type, squares AM type; solid lines October 14 spawning, dotted lines October 10 spawning. See text for explanation of ova types.

Table 2. Test of differences between median times to hatching for pure Glendale (C) ova and Bear-Glendale hybrids (FS + AM) and between FS Bear-Glendale hybrids and AM Bear-Glendale hybrids. See text for description of method of analysis.

		Observed frequencies				Comparisons					
		Cross				All crosses C-F.S.-A.M.		Pure vs. hybrid C - (F.S.+A.M.)		Hybrids F.S.-A.M.	
		Pure	Hybrid								
Trial		C	F.S.	A.M.	Totals	χ^2 total	d.f.	χ^2_1	d.f.	χ^2_2	d.f.
Oct. 10* spawning	Exceeding median	23	51	73	147	53.93^{***}	2	42.34^{***}	1	11.59^{***}	1
	Not exceeding median	77	49	24	150						
	n	100	100	97	297	P<.001		P<.001		P<.001	
Oct. 14* spawning	Exceeding median	8	68	73	149	104.50^{***}	2	104.24^{***}	1	$0.2597^{n.s.}$	1
	Not exceeding median	9	30	27	147						
	n	98	98	100	296	P<.001		P<.001		P=.6103	

*Common median time of hatch for each trial:

October 10: 524.5
October 14: 515.3

Comparisons using combined trials

Pure vs. hybrid

October 10: $\chi^2_2 = 42.34$, $\chi = 6.507$, d.f.=1
October 14: $\chi^2 = 104.24$, $\chi = 10.210$, d.f.=1

Sums: 16.717 2

$$Z = \frac{16.717}{\sqrt{2}} = 11.82^{***} \quad (p=0)$$

Hybrids

October 10: $\chi^2_2 = 11.59$, $\chi = +3.405$, d.f.=1
October 14: $\chi^2 = 0.2597$, $\chi = +0.510$, d.f.=1

Sums: 3.915 2

$$Z = \frac{3.915}{\sqrt{2}} = 2.768^{**} \quad (p=.006)$$

October 14 spawning:

C type: $\bar{x}$ 506.8, med. 507.0 (505.4, 508.8)
FS " : $\bar{x}$ 520.5, med. 519.9 (517.9, 522.0)
AM " : $\bar{x}$ 522.6, med. 523.3 (519.8, 526.8)

For each trial (either October 10 or October 14 fertilization dates) a total chi-square test was performed first among all 3 types; the total chi-square was then partitioned exactly into 2 independent comparisons using the method described by Kimball (1954). The 2 comparisons were pure Glendale versus Bear-Glendale hybrids (C vs [FS + AM]), and FS Bear-Glendale hybrids versus AM Bear-Glendale hybrids (FS vs AM). The results of these 2 comparisons for each trial were then combined using Cochran's (1954) method where chi values for each trial are added, taking into account consistency of direction of the observed differences. The results are shown in Table 2.

Differences in median time to hatching between the pure Glendale and the hybrid types were highly significant for each trial and for the combined trials. In both trials the pure Glendale types hatched significantly earlier than either hybrid, but especially so in the October 14 trial. Differences between the hybrid types were not as great: median time to hatching for the FS cross was significantly less than for the AM cross in the October 10 trial; in the October 14 trial the difference occurred in the same direction but not significantly so. (The combined hybrid trial comparison was highly significant.) The consistently greater median times to hatching for the October 10 trial (Fig. 2) probably reflect experimental rather than genetic sources of variation.

We conclude that differences in times to hatching between the pure Glendale ova and those for Glendale ova fertilized with Bear River sperm represent pronounced genetic differences between the stocks as expressed in the male gene pools, while the lesser differences between hybrid types represent genetic differences related to variability among parents within stocks.

EMERGENCE, MARKING AND RELEASE OF FRY

Because emergence was inhibited intentionally at times by the use of overhead lights, the observed emergence patterns are somewhat artificial. Since fry would emerge only in darkness, lights were used to slow emergence sufficiently to allow marking to keep pace (Daily marking capacity was limited). When lights were used, the same illumination was applied to all boxes containing test group fry.

The numbers of fry which emerged daily, the numbers marked and the numbers of marks deemed "valid" (see MATERIALS AND METHODS: MARKING: Mark evaluation) for each of the 3 types are given in Table 3. (Detailed information about daily emergence per box, numbers marked per box and numbers deemed valid is given in Appendix Table 1.) Figure 3 illustrates the numbers and relative timing of emergence of the test groups (numbers of less than 1,000 fry per day are omitted).

Relative timing of emergence of test groups

Some information about the relative timing of outmigration of the C, FS, and AM test groups of fry in 1976 can be obtained from earlier observations of wild fry migrating downstream from even-year (on-year) Bear River pink broods. Walker and Lister (1971) report the results of operating inclined plane traps 2 consecutive nights each week during fry emigration from Bear River in 1965. They suggest that the run persisted from April 20 to May 24 and that 50% of the outmigration had taken place by May 6 (Fig. 3).

Morley (unpublished data) operated fyke nets suspended from overhead cables from March 20 until May 21, 1975. From traps set sometimes overnight and at other times set and lifted at 1/2 hour intervals throughout the night, he developed an index of abundance of migrating wild fry called "catch per trap per night". Fry were present throughout the period March 20-May 21; recaptures of dye-marked fry showed that the bulk of fry moved from the hatchery site to the river mouth in one night. The catch per trap per night figures are shown on Fig. 3.

The numbers of each type of test fry emerging daily from the incubation boxes in 1976 are shown also on Fig. 3. (Numbers of less than 1000 per day are omitted at the start and end of emergence). The figures suggest that the 50% points of emigration of test fry preceded those observed for on-year fry by 1 to 3 wk. The C type fry (of pure Glendale origin) migrated earliest, while the hybrid FS and AM types were intermediate to the early C type and those observed from natural on-year runs. We noted earlier in this report that the C type fry had hatched earlier than the FS and AM types.

We conclude that the relatively early outmigration of the pure Glendale fry, and the intermediate timing of the hybrid types reflects an inherent tendency for the Glendale eggs and fry to develop, hatch and emerge more rapidly and earlier than Bear River eggs and fry.

Releases of unmarked fry

The only unmarked fry released arose from Glendale × Glendale fertilizations (see MATERIALS AND METHODS: TEST DESIGN). In total, these amounted to 1,233,569.

An initial release of 225,697 stack-incubated fry arising from early egg-takes was made on March 31. They appeared to be at the appropriate stage of development for release, although the emergence of fry incubating in gravel boxes had not yet begun.

The remaining 1,007,872 unmarked fry were released from 'density-study' gravel boxes as they emerged (633,294), from hatchery stacks ('Astro-turf study' 86,604; surplus 392,275), or with test groups released daily (surplus C type fry remaining after marking). Releases (excluding the March 31 release) occurred from about April 15. Fry from the density-study gravel boxes appeared to be in good condition; the Astro-turf study fry were at an advanced developmental stage with probably small chance of survival. Other stack fry were probably developed slightly beyond the optimum release stage but were still in good condition.

Table 3. Numbers of test group fry which emerged daily from incubation boxes, numbers marked, and numbers of marks deemed "valid" (see text). Figures are totals for the two boxes of each type. (Figures for individual boxes are given in Appendix Table 1.) All marked fish were marked and released on day of emergence except where indicated.

Date	C type			AM type			FS type		
	No. emerged	No. marked	"Valid" marks	No. emerged	No. marked	"Valid" marks	No. emerged	No. marked	"Valid" marks
Apr. 9[a]	584			40			151		
10	1248			14			45		
11	2296			15			87		
12	35434	19594	17151[b]	156			1221		
13	43549	16263	15557	879			4490		
14	26077	18374	17812	1308			3805		
15	34464	9820	9820	6491			10811	8118	8118
16	27922	17630	16656[c]	11313	5382	5285	17950	5945	5759
17	6307	6069	5629	3837	3468	3429	5264	4981	4805
18	5366	5154	4813	4668	3184	3184	6407		
19	8291	7975	7803	7989	6893	6893	8718	8396	8120
20	14012	13478	13030	17536	7371	7284	18450	11538	10530
21	21687	13559	12648	39979	3059	3023	40146	18726	17298[d]
22	4725	4452	4281	14752	13303	13004	10842	10449	10401
23	3625			19363	15735	15735	12230	11573	11573
24	5515			23156	15620	15620	19594	14075	14075
25	6329	3228	3032	30637	5203	5203	27725	6119	6119
26	6229			23794	20061	19740	20937	12655	12489
27	5135			21826	20936	20423	19363	14570	14570
28	4314			24009	15422	15422	19746	8902	8684
29	1610			6901	379	379[e]	6196		
30	843			4817			5542		
May 1	533			3130			3743		
2	142			1253			1676		
3-13[f]	326			824			1236		
Totals	266563	135596	128232	268687	136016	134624	266375	136047	132541

[a] Total emergence to this date inclusive.
[b] 1829 marked and released April 13.
[c] 5392 marked and released April 17.
[d] 7868 marked and released April 22.
[e] Marked and released April 28 but considered part of April 29 emergence.
[f] Last observations May 13; emergence from any box less than 10/day.

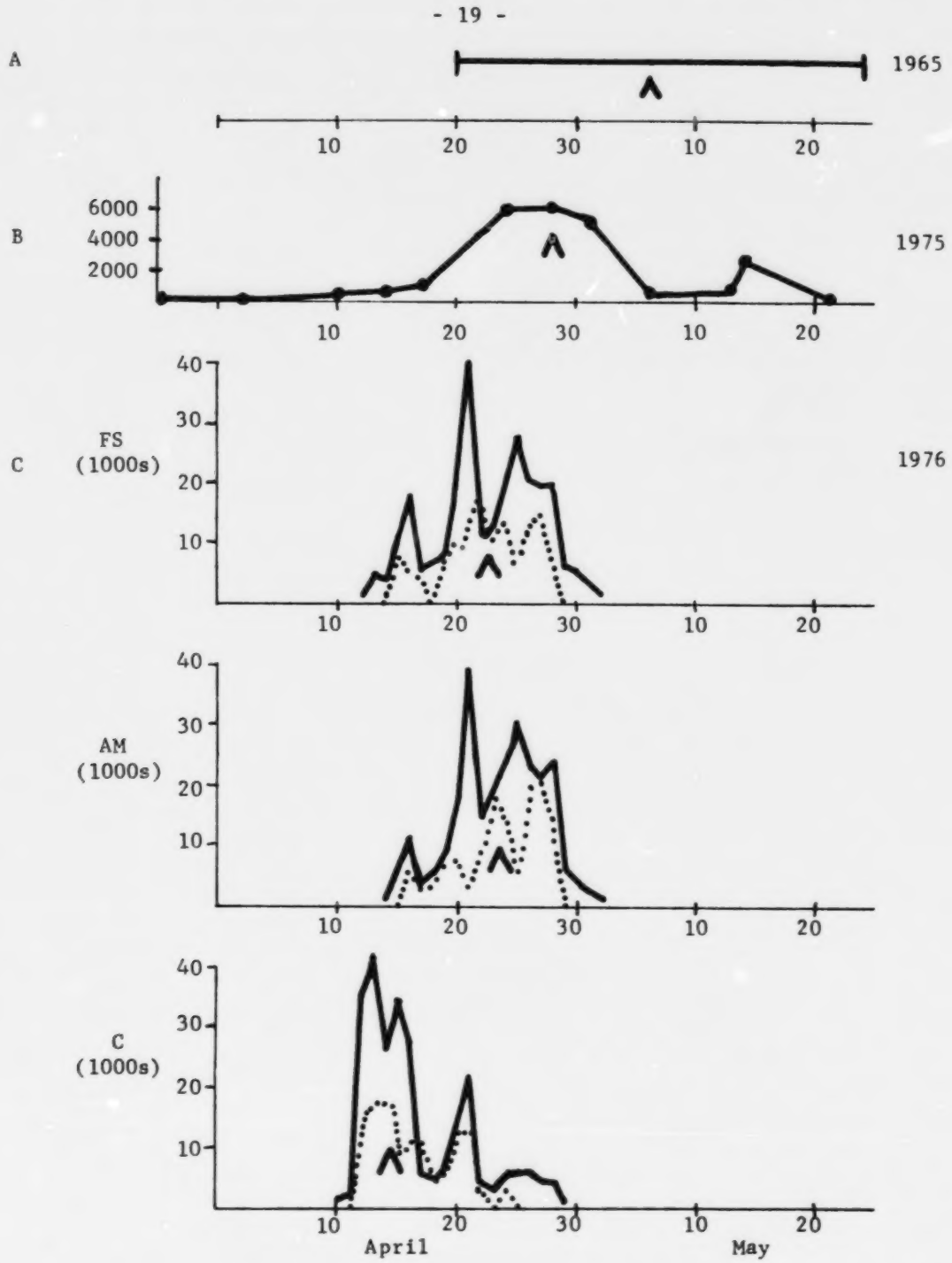

Fig. 3. Outmigration of Bear River pink fry. A. Duration of natural fry run in 1965 as indicated by catches from inclined plane traps, and estimated 50% point of fry migration (arrow) (from Walker and Lister 1971). B. Estimated catch per trap per night in fyke nets in 1975 and time of migration of natural fry run (R.B.M., unpublished data). C. Daily numbers of test group fry emerging from incubation boxes (solid line) and numbers of validly marked fry released (dotted line) in 1976 (from Table 3).

The reason for releasing these unmarked fry was to buffer marked fry against excessive predation. Because the C type fry emerged earlier they may have received more benefit, if any, from the buffering effect.

Marking and release of test fry

Test group fry were marked by fin-clipping during the day following their emergence from the gravel boxes. Marked fry were counted as they were fin-clipped and those which were surplus to each day's requirements for marking were destroyed after being counted (except C type fry which were released). Table 3 shows the numbers of each type emerging each day, the numbers marked, and the numbers considered to be valid marks of the required type.

Because C type fry emerged earlier, marking began first on this group and was concluded first. The emergence of the FS and AM types was similar, so the pattern of marking of these types tended to be parallel. Approximately 50% of the total emergence from each of the 6 test boxes was marked (Table 3, Appendix Table 1).

Mark quality was determined by frequent inspection of fry fin-clipped by each marker (see MATERIAL AND METHODS: MARKING: Mark evaluation). The best mark in terms of both proportion of valid marks and mark quality was the BV. Results were as follows:

Test group	Mark	Estimated % invalid	Weighted av. score
C	AdLV	5.4	55.2
AM	BV	1.0	58.6
FS	AdRV	2.6	55.5

Perfect score = 60.

A non-parametric Friedman 2-way analysis of variance test (Siegel 1956, p. 166) using each marker's ranked mark score averages showed that there was a significant difference in mark quality ($p < .01$) due almost entirely to consistently higher ranking for the BV mark (9 out of 10 of the markers scored best on this mark). The remaining 2 marks ranked about equally.

LENGTHS, WEIGHTS, AND DEVELOPMENTAL INDICES OF EMERGENT FRY

Test fry emerged from the incubation boxes from April 10 to May 3. Fifty fry were collected from each box each day, preserved in formalin, and later measured and weighed. (See MATERIALS AND METHODS: EMERGENCE). Mean lengths, weights and K_D values for the daily samples are given in Appendix Table 2. Since our intent was to compare average values among entire emerging populations from incubation boxes, means for the pooled daily samples from each box (approx. 1,000 fry) were calculated. Because of changes over time in lengths and weights (Appendix Table 2) and in numbers emerging (Fig. 3) a second set of means based on daily samples weighted to daily emergence was

calculated according to the method described by Cochran (1963, p. 87). Both sets of means, their estimated standard errors and confidence limits are given in Appendix Table 3. As suspected, weighting had a pronounced effect on the estimates of population means for the Control (C) fry, because a large proportion had emerged in a relatively short time. We concluded that the pooled sample means were not representative of all the populations and should not be used for making comparisons. Because we are unaware of methods for making statistically valid comparisons using weighted means, single samples of each box's total population were obtained by randomly sub-sampling each daily sample of 50 fry in numbers approximately proportional to the numbers which had emerged that day, to give total sub-samples of 200. Exact details of this method are given in Appendix C. Means of these sub-samples are also shown in Appendix Table 3. Their similarity to the weighted means supports the validity of this approach.

The sub-samples of about 200 were examined for differences in central tendency using the simultaneous test procedure (STP) method described by Sokal and Rohlf (1969) which is based on U, the Willcoxin-Mann-Whitney statistic (a non-parametric test was chosen because the sample distributions were skewed). In the STP test, significant differences don't necessarily indicate a difference in means, only that the bulk of individuals in one population is different from the that in another. Results of this analysis are shown in Fig. 4. The sample means are arranged in order of increasing magnitude, and statistically homogeneous groupings as defined above ($\alpha = .05$) are underlined.

The test shows that replicate pairs within any one type of emerging fry were not statistically different in length, weight or stage of development (K_D). In length, both AM (Accelerated Male) replicates were significantly distinct from the C (Control) replicates while FS (Frozen Sperm) replicates were intermediate. In weight, the only significant difference was between one FS replicate and one AM replicate, while the 4 remaining replicates were statistically homogeneous. With respect to stage of development, both C replicates were significantly greater than the AM replicates in K_D, while the FS replicates were intermediate. Thus the C fry were shorter and less developed at emergence than the AM fry.

Although the FS fry were not statistically distinct from the AM fry, the fact that AM fry were distinct from C fry while the FS fry were not suggests that there was a difference between AM and FS fry for these characters. We cannot explain these inferred differences, in view of the fact that the male components were both derived from the 1974 Bear River run. Of course the males which provided the frozen sperm for the FS type fry were not the same individuals as those used to fertilize the ova which produced the accelerated males for AM type fry. It is possible that there was unintentional selection from among the males used to provide the two types of milt from the 1974 run, or that only certain genetic types among the accelerated male stock became mature in the fall of 1975 and hence only these could be used to fertilize the test ova.

SURVIVALS TO EMERGENCE

Survivals of the 3 test groups can be estimated from records of live and dead eggs and fry maintained from the time of fertilization until

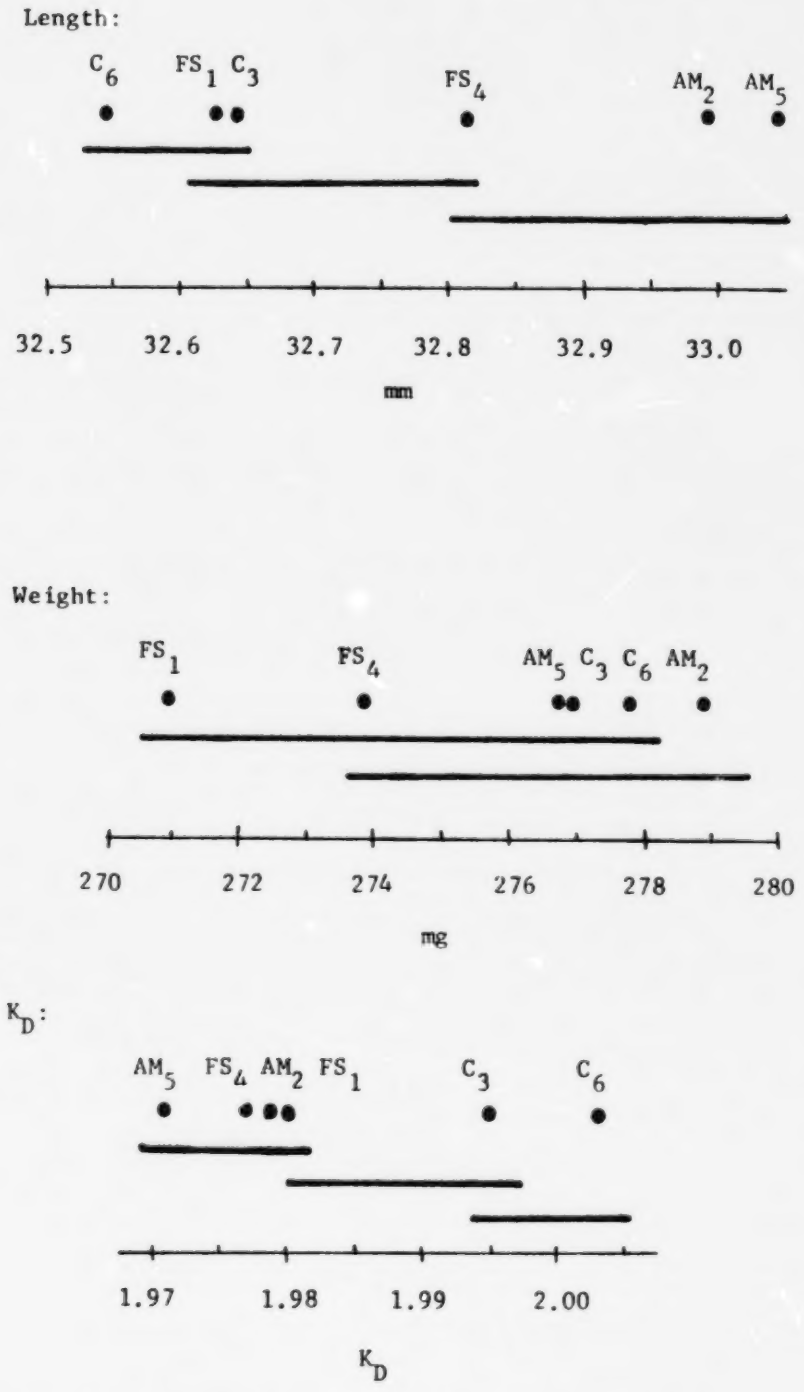

Fig. 4. Average lengths, weights and development indices (K_D) for C (Glendale ♂ × Glendale ♀), AM (accelerated Bear River ♂ × Glendale ♀) and FS (frozen Bear River sperm ♂ × Glendale ♀) types of fry emerging from different incubation boxes (subscripts 1-6). Statistically homogeneous groupings of emerging populations are underlined. See text for explanation of statistical method.

emergence from the upwelling gravel boxes. For each group (C, FS or AM) the numbers of ova to which milt was applied can be determined by adding the numbers of dead eggs picked and counted at the eyed stage and at the time of planting in the gravel boxes, to the numbers of remaining live eggs estimated volumetrically (Appendix B). The estimates of numbers of eggs planted were also based on these volumetric determinations. The number of fry emerging from each gravel box was determined by adding the number marked to the unmarked surplus from each box counted and destroyed. The numbers of eggs and fry alive at each stage and the estimated survivals are shown in Table 4.

Survival of the C and AM groups to the eyed and emergent stages was high and nearly the same. The somewhat higher survival of the AM group to the eyed stage might be ascribed to differences in length of storage of the C and AM milt prior to application to the ova. (C milt was collected at the Glendale River, stored in chilled glass jars and flown to Bear River where it was applied to the test ova several hours later. AM milt, on the other hand, was collected just prior to fertilization and had thus been stored only a short time before application.)

Survival of the FS group to the eyed stage was about one-half that of the C and AM groups. (The frozen milt had been collected on the Bear River 1 year before and retained in liquid N_2. It was thawed at the time of application.) In view of the level of the technology of fish sperm cryopreservation at the time and the fact that the application was on a production scale outside of a laboratory, the fertilization rate achieved (over 45%) must be considered exceptional (see Horton and Ott 1976). Microscopic observation of the dead FS eggs revealed that most of them had not been fertilized, rather than having died after fertilization. Subsequent survival of the remaining live, eyed FS eggs was comparable to that of the C and AM groups.

RECOVERIES OF MARKED FISH

Commercial fishery

About 22% of the total 1977 catch of pink salmon from the 3 major fisheries in southern B.C. (exclusive of the west coast of Vancouver Island) was examined for missing fins when the fish were delivered to processing plants. Thirty-one percent of the catch from Johnstone Strait, the major fishing area adjacent to Bear River, was examined, as were as 6-7% of the catches from Juan de Fuca Strait and the Fraser River estuary area (Table 5, Fig. 1).

A total of 126 pinks whose missing fins would classify them as belonging to the 3 test groups was recovered, representing an overall ratio of 1 marked:5209 unmarked fish. The ratio among fish examined from Johnstone Strait catches was 1:4968 and from Juan de Fuca Strait catches 1:6231. The differences in these ratios is not significant (χ^2 = 0.48, 1 df; p. 0.49). No fish with fins missing in the combinations employed in the test were found among some 18,000 pinks examined from the Fraser River fishery. We conclude that returning test fish approached the inside waters of southern B.C. both from the north (Queen Charlotte Strait) and from the south (Juan de Fuca).

Table 4. Survivals of test groups to emergence.

Test group	Survival to eyed stage[a]			Survival in gravel boxes				
	Ova to which milt applied	Live at eyed stage	% survival	Box no.	Eyed eggs planted	Emergent fry	% survival	% survival overall
Control (C)	365,018	335,060	91.8	3	136,523	132,365	97.0	89.0
				6	136,359	134,198	98.4	90.3
				3+6	272,882	266,563	97.7	89.7
Accelerated male (AM)	367,639	351,281	95.6	2	136,784	135,022	98.7	94.3
				5	136,587	133,665	97.9	93.5
				2+5	273,371	268,687	98.3	93.9
Frozen sperm (FS)	600,980	271,975	45.3	1	135,948	132,968	97.8	44.3
				4	136,027	133,407	98.1	44.4
				1+4	271,975	266,375	97.9	44.3

[a]Non-survivors included both unfertilized and dead fertilized eggs.

Table 5. Weekly pink salmon catches (seine, gillnet, and troll combined), numbers of individuals examined weekly for missing fins, and numbers of marked fish recovered from major southern B.C. pink fisheries in 1977. Numbers of fish with missing or reduced fins other than those combinations employed for the test are shown also.

Week ending	Catch	Number examined	Test marks			Other marks			
			AdRV (FS)	AdLV (C)	BV (AM)	Ad	RV	LV	AdBV
		Johnstone Strait (Areas 12 and 13)[a]:							
To Jul 16	9,113								
Jul 23	64,060	20,118		1	2	2	10	7	
Jul 30	108,057	47,336			4	14	18	22	
Aug 06	141,436	47,782	4	1	1	33	8	4	
Aug 13	31,131[b]								
Aug 20	246,702	73,040	15	11	13	37	9	5	
Aug 27	732,661	211,229	7	9	6	84	17	11	
Sept 03	355,973	107,433	3	10	6	39	10	8	
Sept 10	114,307	47,984	2	9	4	25	3	7	1
Sept 17	27,642	12,548		5	1	3	1		
Sept 24	15,813	8,803		2		2	2		
After Sept 24	608								
Sub-totals	1,847,503	576,273	31	48	37	239	78	64	1
		Juan de Fuca Strait (Area 20):							
To Jul 30	5,040								
Aug 06	18,779	97							
Aug 13	79,121	8,997				1	1	1	
Aug 20	355,136	23,773			1	7	1		
Aug 27	228,026	11,403				5		1	
Sept 03	128,513	7,249				5			
Sept 10	47,732	7,740		7	1	1	2	3	
Sept 17	10,362	2,923		1		6		1	
Sept 24	674	132							
After Sept 24	60								
Sub-totals	873,443	62,314	0	8	2	25	4	6	0

Table 5 (cont'd)

Week ending	Catch	Number examined	Test marks AdRV (FS)	Test marks AdLV (C)	Test marks BV (AM)	Other marks Ad	Other marks RV	Other marks LV	Other marks AdBV
			Fraser River Area						
To Sept 10	207,787								
Sept 17	69,374	14,566				5	1		
Sept 24	15,099	3,224				2			
After Sept 24	2,145								
Sub-totals	294,405	17,790	0	0	0	7	1	0	0
Totals	3,015,351	656,377	31	56	39	271	83	70	1

[a]Of the 112 packer loads of pinks examined from the Johnstone Strait fishery, 100 contained fish from Areas 12 and 13 only. The remaining 12 loads included small numbers of fish from one or more of Areas 6-11 and 16. All but 2 of the test marks were recovered from loads picked up in Areas 12 and 13 exclusively; 1 BV and 1 AdRV mark were recovered from loads containing some fish from Areas 9-11.

[b]Troll-caught fish only; net fishery closed.

Within the northern approach, Area 12 includes Queen Charlotte Strait and the northern portion of Johnstone Strait, while Area 13 contains the remaining southern portion of Johnstone Strait (Fig. 1). Glendale River and its approach through Knight Inlet lie in Area 12, and Bear River lies within Area 13. Pinks examined from packer loads collected exclusively from Area 12 numbered 275,875, among which 53 test-marked fish were found (1:5202). Those examined from loads delivered from Area 13 numbered 157,527 from which 45 marks were recovered (1:3501), suggesting that pinks arising from the test were more concentrated in Area 13 adjacent to Bear River (χ^2 = 3.9, 1 df; p. 0.048). One packer load of 10,016 pinks from Knox Bay (6 km from Bear River) and Quathiaski Cove (32 km) contained 15 test-marked fish (1:668). These observations suggest that at least some of the test-marked fish tended to assemble near the mouth of Bear River.

Stream recoveries

On Bear River, the counting fence at the head of tide was operated from July 8 until October 22, 1977, when a freshet washed out a section of panels. In on years almost all pinks would have entered the stream by this latter date (D.F.O. annual reports of salmon streams and spawning grounds, and the authors' observations in 1972, 1974, and 1976). Up to that time the total count was:

Chinooks	19	Sockeye	1
Coho	85	Dolly Varden	158
Chums	11	Rainbows	3
Pinks	1		

The lone pink was a male, unmarked.

During the period of fence operation, the Bear River estuary and the immediately adjacent portion of Johnstone Strait were surveyed for signs of pink salmon, live or dead. None was seen. After the washout, the river was surveyed whenever weather conditions favored good visibility: several live and dead salmon and steelhead were seen, but no pinks. We must assume that only 1 pink salmon entered the Bear River in the fall of 1977.

The Glendale River (source of the parents which provided the ova and control milt used in the test) was surveyed 5 times between July 5 and October 12, 1977. Opportunity to see and recover whole carcasses was good. Pinks were first observed in the river on August 9. From October 4 to October 12, 3,662 spawned out, dead pinks were examined. Four lacked pelvic fins, 3 lacked right pelvics. None was found with missing fins in the pattern employed in the test. We conclude that few, if any, of the test fish entered Glendale River.

Several small streams entering Johnstone Strait between Camp Point (7 km west of Bear River) and Deepwater Bay (23 km southeast) were surveyed between September 8 and October 16, 1977. No pinks were observed in any of them.

Comparison of recovered test groups

In assessing the relative return of test fish, only the AdRV (Frozen Sperm) and AdLV (Control) recoveries were compared because of the likelihood of bias in recovering BV (Accelerated Male) types and possible greater natural incidence of this mark (see MATERIALS AND METHODS: ADULT RECOVERIES: Commercial fishery, and MARKING: Marks used). Overall, in the Johnstone Strait and Juan de Fuca Strait fisheries, 87 AdRV and AdLV marked salmon were recovered from among 638,587 examined. Thirty-one of the marks were AdRV and 56 AdLV. This difference is highly significant (χ^2 = 8.0, 1 df; p. 0.005). The recovery of AdLV marks was also greater than that of AdRV marks in the Johnstone Strait fishery (Table 5) (χ^2 = 0.038, 1 df; p. .05). We conclude that Control pinks either survived better or strayed less than did the Frozen Sperm type.

Wherever possible lengths, weights and sex of recovered marked pinks were recorded. Comparison of males and females of the AdRV and AdLV types shows that female Control pinks were heavier (16%) and longer (7%) than Frozen Sperm fish. Males were not significantly different (Table 6). Parentage of the two types differed only in respect to males: milt used to produce the Frozen Sperm type was collected from 95 males (post-orbital-hypural length $\bar{x}$ = 41.8 cm, SD 3.40) of the 1974 Bear River spawning run, whereas that used to produce the Control Type was taken from 590 Glendale males in 1975, a sample of 122 of which measured on average 44.4 cm, SD 3.11. This difference in size of male parents is highly significant (t = 5.73, 215 df; p < .001) and may have been reflected in the size of the progeny. We have noted earlier that male pink parents exert a strong positive influence on embryonic growth (Withler and Morley 1970), but cannot explain why the difference in size of progeny in this case was expressed only among females.

Among the combined AdRV and AdLV recoveries whose sex was recorded, there were 28 males and 53 females, a significant deviation from an expected 50:50 ratio (χ^2 = 7.716, 1 df; p 0.005). Such differences in sex ratio in commercial catches of pinks have been observed before (e.g. Bams 1973) and ascribed to selection by fishing gear in favor of females.

DISCUSSION

The design of this experiment depended heavily upon a commonly observed phenomenon. In the past, when pink salmon eggs had been transplanted into a stream which once supported pink spawners, or into the off year of one whose runs had a pronounced on-off condition, there were often substantial returns to the receiving stream. A good example is provided by the transplants of a total of some 12.6 million eggs to the Big Qualicum River on the east coast of Vancouver Island (Walker and Lister 1971). Nearly 6 million eggs collected from the Cheakamus River in 1963 and planted in the Big Qualicum produced an escapement of only 100 fish in 1965, no different from that of previous years. But in 1966, 11,940 pinks returned from a planting of nearly 7 million eggs from the 1964 Bear River run. After spawning naturally,

Table 6. Comparisons of average lengths and round weights of AdRV- and AdLV-marked pink salmon recovered from catches in Johnstone Strait and Juan de Fuca Strait fisheries, 1977. Lengths were measured from the posterior edge of the orbit to the hypural plate.

Character	Comparison		AdRV (FS)	AdLV (C)	t
Weight (kg)	♀ vs. ♀	$\bar{x}$	2.00	2.32	0.321**
		SD	0.282	0.322	
		n	20	31	
	♂ vs. ♂	$\bar{x}$	2.25	2.36	0.115
		SD	0.347	0.647	
		n	10	17	
Length (cm)	♀ vs. ♀	$\bar{x}$	43.99	47.11	3.122**
		SD	2.982	2.746	
		n	21	32	
	♂ vs. ♂	$\bar{x}$	45.51	46.82	1.312
		SD	1.917	3.76	
		n	10	18	

these in turn produced 3,000 spawners in 1968, which in turn produced 300 in 1970 (the run subsequently petered out entirely). Similarly, a series of transplants into Robertson Creek, an outlet of Great Central Lake which had not supported pinks formerly, produced small but consistent returns to the stream (100 to 240); in 1963 over 4,700 returned to the stream and nearby (Neave 1965).

Also, in an attempt to establish a run in an off-year, in 1954 2.6 million eyed eggs from the Lakelse River in the Skeena River watershed of central B.C. were planted at Jones (Wahleach) Creek, a lower river tributary of the Fraser watershed. About 2,800 adults returned to the stream in 1956 and deposited an estimated 2 million eggs which were supplemented by a further 1 million from the Lakelse. The return in 1958 was about 100 adults, and only a few were seen in 1960.

Of course not all transplants of pinks had produced even modest returns. Those made into McClinton Creek (on Graham Island, Queen Charlotte Islands) from the 1931, 1933, and 1935 brood year runs of the Tlell River produced less than 10 returns each; many of the early transplants from Alaskan rivers into Puget Sound streams in their off-years apparently produced no returns until methods for rearing fry in saltwater impoundments before release were employed. Even then, the returns were small (Neave 1965, Withler 1982).

Nevertheless, evidence from past transplants suggested that there was a substantial likelihood that the pure Glendale fry used in the experiment would produce enough returns to serve as controls against which to assess the return of treated fish (the AM and FS fry). When in fact no Control pinks returned the experiment was put in jeopardy, although not completely so if some of the AM and FS pinks had returned to Bear River. When none of these returned either, the critical test of the hypothesis was completely invalidated.

In designing the experiment we had assumed that (1) we would be able to mark and release about 250,000 live fry of each test group, (2) the overall survival of marked fry from release to return as adults might be 0.005 and (3) the fishery might remove as many as 75% of the returning fish before the remainder entered Bear River. On the basis of earlier observations we considered a 0.005 survival of marked fry to be conservative (e.g. see Bams 1976, Parker 1962), and that a fishery removal rate of 75% would be rather high. In practice we released only about 130,000 of each test group. On the basis of assumptions (2) and (3) we might have expected some 160 pinks of each group to return to the river after some 490 had been caught. Projecting the recoveries of marked fish among the samples from the fishery to what might have been present in the entire Johnstone Strait and Juan de Fuca catches, it appears that there might have been as many as 250 or so of the AdLV (Control) test group caught. If so, and assuming the fishing rate to have been about 75%, the total returning number of adults from the AdLV test group might in fact have been some 330 or so, above half that projected. It should be noted that the 1977 pink escapements to the Johnstone Strait area as a whole were "the second lowest on recent record" and that the catches were some 200,000 less than expected (Anderson and McKinnon 1979), suggesting that survivals of pinks of the 1975 broods from that area were generally poor.

In spite of the indicated poor survival, we have shown from sampling the fishery catches that test group pinks tended to congregate in the vicinity of Bear River. Why these failed to enter the river itself we cannot tell. It seems highly unlikely that the Johnstone Strait fishery, intense though it may have been, removed every last one. Even more remarkable perhaps was the failure of the non-test fry to return. It will be recalled that some 1.2 million unmarked fry also were released, partly as buffers against predation on the daily releases of marked test group fry. At a conservative survival rate for unmarked fry of 0.01 (Bams 1976, Parker 1962) some 12,000 of these fish could have returned as adults. (They would not have been detectable in fishery catches of course.) With a 75% fishery 9,000 might have been caught, leaving up to 3,000 to enter streams. As we have seen, only 1 unmarked pink entered Bear River in 1977 and nearby streams contained few or no pinks. We have no satisfactory explanation for the failure of either marked or unmarked pinks to return to Bear River or nearby streams.

While the experiment failed to throw any light on the main hypothesis (i.e. that infusion of on-year home stream male genes into the eggs of a donor stock introduced in the off-year cycle would improve the return of adults over that from entirely foreign eggs), it did demonstrate that two techniques of transferring genetic material from one pink cycle to the other could be applied on a production scale. The experiment had been conceived in the early 1970s and the problem of transferring genes between cycles had been addressed immediately in two ways. One approach was to accelerate maturation of on-year juveniles by regular injections of salmon gonadotropin extracted from pituitary glands of maturing salmon, and also (later) to accelerate growth by incubating eggs and rearing juveniles at elevated temperatures. Relatively large, fertile males exactly one year old were produced for the experiment (Donaldson et al. 1972, McKinnon and Donaldson 1978). At the same time work had begun on methods of salmon sperm cryopreservation, by adapting and improving earlier techniques (Horton et al. 1967). By the mid-1970s the fertilization rates of cryopreserved pink salmon sperm had reached levels which also warranted application (Horton and Ott 1976). We have seen from the results that both techniques (acceleration of maturity, cryogenic sperm storage) can be used on a large scale to provide excellent, or at least acceptable, fertilization rates of ova, and normal-appearing fry which survive to the adult stage.

The hypothetical basis for the experiment inferred that there are genetic differences between pink salmon stocks which influence their ability to home to their natal streams. The experiment failed to demonstrate whether or not the inference regarding homing is sound, but it did show clearly that genetic differences existed between the Glendale and Bear River stocks. The pure Glendale fry type hatched earlier and emerged earlier (and hence would have migrated earlier), than the hybrid types. Such differences would have been useful in interpreting the reason for disparities in rates of homing to the Bear River, had any of the test groups returned.

The question of what mechanism(s) have initiated and perpetuated the phenomenon of on-offness in certain pink salmon rivers remains unanswered, although several hypotheses have been forwarded to account for its perpetuation once established (Ricker 1962). None of these seriously considers the effect that genetic differences between the odd- and even-year lines may have had on disparity in abundance between the lines. Yet the life

history characteristic of invariable maturity at age 2 (and consequent absence of male precocity) in their native range most clearly sets the pink apart from other Pacific salmon, in that it imposes complete genetic isolation between the lines. There is enough evidence of consistent phenotypic difference between the two to infer that there are substantial genetic differences between them. On the North American coast, for example, odd-year pinks caught in the fisheries have been larger on average than even-year fish since the fisheries began, in spite of great differences over the years in fishing intensity and the type of gear used for harvesting, and marked differences in absolute abundance (Hoar 1951, Godfrey 1959, Ricker 1972, Ricker et al. 1978). A difference in ova size between odd- and even-year lines occupying the same spawning ground on the Babine River has been noted (Bilton and Smith 1973), as well as a consistent difference in time of spawning (H. D. Smith personal communication). Greater differences in frequency of certain polymorphic enzymes among Alaska pink stocks have been observed between the lines occupying one stream than between stocks occupying geographically separated streams within one or other line (Aspinwall 1974). Regarding Asian stocks Dyagilev and Markevich (1979) have suggested that the relative success of transplants of eyed eggs to the Kola Peninsula from odd-year Sakhalin runs as compared to the indifferent success of even-year transplants may be attributed to the fact that the odd-year line spawns earlier (August-September) than does the even-year line (September-October). Thus, in the colder Kola Peninsula streams, the developing odd-year eggs have passed the stage where they are susceptible to death or malformation from low temperatures by the time river temperatures drop to less than 4-5°C, while the even-year eggs have not.

Aspinwall (1974) suggests that the greater differences between lines than within them may have arisen from different origins of the lines. Genetically-biased samples may have flowed out from different refugia created by the Cordilleran ice sheet of the Wisconsin glacial period and occupied streams and rivers which became available as the ice receded (Withler 1982). It could be hypothesized, for example, that the massively dominant odd-year line occupied the Fraser River and Puget Sound streams from the southern salmon refuguim (McPhail and Lindsey 1970) because the stock(s) possessed the genetic fabric appropriate to freshwater conditions of that area, whereas the dominant even-year line now occupying Queen Charlotte Islands watersheds might have arisen from the Alaskan-Yukon refugium whose stocks were genetically pre-adapted to conditions in that area. In other areas, such as central B.C. and southeast Alaskan watersheds, pinks of either origin might have found the watersheds habitable and both lines would be represented with appearances of partial dominance, or none. Of course, one or both lines could have arisen from one or more other discreet refugia such as the Bering Sea land bridge or the unglaciated watersheds of the eastern Asian coast.

Whatever the origin of present day western North American pink stocks, the hypothesis that odd- and even-year lines represent genetically biased founding stocks unequally suited to various environments (probably freshwater [Aspinall 1974]), best satisfies the questions both of the initiation and the perpetuation of on-offness.

Results of the experiment reported here can only be called inconclusive. However, from a practical point of view, and until a more supportable hypothesis is constructed, it will be appropriate to attempt to

transfer the genetic complement of successful on-year lines into the barren off year of pink-producing streams, for the purpose of exploiting the vast potential of spawning area currently barren of pinks in one year out of two.

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APPENDIX A. COLLECTION AND FREEZING OF CRYOPRESERVED SPERM, 1974

COLLECTION

One hundred fifteen ripe male pinks were collected from the Bear River by beach seine between October 12 and 25. These were backpacked 4-5 at a time in a mixture of ice and water in large plastic bags from the stream to a logging road about 1 km from the capture site. They were then placed in a large tank containing aerated water and ice on the back of a pickup truck and driven to the Pacific Biological Station. There they were transferred to a large holding tank and held until stripped.

Over the period October 17-31, 95 males were selected for stripping. These fish supplied all of the milt used to fertilize the frozen sperm (FS) ova used in the experiment. Their average fork length was 54.8 cm, SD 4.80 cm; their average post-orbital-hypural length was 41.8 cm, SD 3.40 cm.

Prior to stripping, each male was killed by a sharp blow to the snout and wiped dry with paper towels. Milt was collected in 250 mL glass beakers as it was expressed from the male by gentle pressure. The milt of 3-6 males was combined before dilution in the diluent.

FREEZING

The diluent was composed of an extender and a protective agent. The extender chosen was 275, one of several developed by Ott (1975). It was prepared by adding 7.65 g NaCl, 3.38 g $NaHCO_3$ and 6.75 g vegetable lecithin to 900 mL distilled water. Dimethyl sulfoxide (DMSO) was used as a protective agent; 100 mL was added to the extender as prepared above, to make diluent batches of 1000 mL each. The proportion of DMSO in the diluent was thus 10%.

Each portion of pooled milt was mixed with diluent in the ratio 4 parts diluent: 1 part milt. The mixture was drawn into a large syringe and distributed in 2 mL portions into 6 oz. (177 mL) Whirlpak plastic bags from which the closing device had been trimmed off. Excess air was pressed from the plastic bag and the open end heat-sealed in a Daisy Seal-a-Meal plastic bag sealer, model 5600. Sealed bags were placed immediately in liquid nitrogen (LN_2) vapor generated in a Minnesota Valley Engineering Cryogenics vapor freezing unit model CBF21. After about 10 min, by which time the milt-diluent mixture was frozen hard, the plastic bags were immersed in LN_2 in storage containers. Two kinds were used: Cryenco Biostat Container model 3000 and Linde model LD30. The containers were topped up with LN_2 weekly until the frozen milt was used approximately 12 mo later. Thirty-one hundred 2 mL packets were prepared and frozen between October 16 and 30. These formed the entire bank of frozen sperm used in the experiment.

APPENDIX B. DETERMINATION OF EGG NUMBER BY VOLUME

The numbers of eggs surviving up to planting were determined volumetrically. After the eggs were fully eyed (300°C days) each Heath incubator tray containing eggs was removed from its stack and allowed to drain. The eggs were sprayed with a strong water jet, after which the dead eggs were removed and counted. The volume of remaining live eggs was measured in water in plastic 32 fl oz (950 mL) graduates.

A series of counts were made of eggs in measured volumes. The samples were drawn from groups taken from Glendale spawners over a range of spawning dates as follows:

Spawning date	Volume of eggs (fl. oz)	No. eggs	No. eggs per fl. oz.
Oct. 6	36 1/4	3855	106.3
6	28	2912	104.0
8	22	2210	100.4
9	32	3507	109.6
10	32	3531	110.3
14	32	3537	110.5
15	32	3630	113.4
16	32	3474	108.6
17	32	3506	109.6
18	32	3728	116.5

On the basis of these observations we determined that the number of eggs per fl. oz. was 109 ± 2.99%. This figure was applied in determining the numbers of live eggs remaining in the incubator trays after picking, and subsequently the number of eggs of each test group planted in incubation boxes.

APPENDIX C. SELECTION OF RANDOM SUBSAMPLES FOR LENGTH, WEIGHT AND K_D ANALYSIS

The subsamples were selected randomly using a 2-step technique to simulate what would have happened had the total population been sampled at one time. The probability of randomly selecting a particular fish from the total population is (by definition):

$\frac{1}{N}$, where N equals the population (total number emerging from an incubation box).

This probability can be expressed as:

$\frac{1}{N} = \frac{n_i}{N} \times \frac{1}{n_i}$, where N is again total emergence from a box and n_i is the emergence from that box on day i.

This expression means that the probability of selecting a particular fish is the probability of selecting a fish on a particular day times the probability of selecting a particular fish on that day. In a truly random sample the n_i/N values would vary randomly about n_i/N (in contrast to a strictly proportional sample where they would be fixed).

The procedure employed in selecting the subsample was first to select at random the n_i/N values for each day. These values were then used to determine the number of records to be selected from each previously weighed and measured daily random sample of 50 to give the desired total sample of 200.

The n_i/N values were determined by first expressing total emergence on a cumulative linear scale, e.g., for a theoretical total emergence of 100,000 over a period of 5 days:

Cumulative emergence	0	20,000	35,000	60,000	85,000	100,000
(Interval/day)	0	1	2	3	4	5

To determine the n_i/N values for a random sample of 200, 200 random numbers between 1 and 100,000 would be generated, assigned to the correct day, and tabulated in a frequency distribution over this linear emergence scale. The relative frequency for each day can then be used to obtain random estimates for each $n_i/N \times 200$, the number of records to select from the random sample of 50 for day i. (These frequencies were found to vary slightly from frequencies calculated in direct proportion to emergence, as would be expected).

The required number of records from each daily random sample of 50 was then selected using random numbers, each fish in the daily sample of 50 theoretically having an equal probability of $1/n_i$ of being selected from the total emergence for that day.

Appendix Table 1. Numbers of test group fry which emerged daily from incubation boxes, numbers marked, and numbers of marks deemed "valid" (see text) by box. All marked fish marked and released on day of emergence, except where indicated.

TYPE C

Date	Box 3			Box 6		
	No. emerged	No. marked	"Valid" marks	No. emerged	No. marked	"Valid" marks
Apr 9[a]	80			504		
10	154			1094		
11	327			1969		
12	5706	3290	2851	29728	16304	14300[b]
13	13542	2161	2067	30007	14102	13490
14	11285	10957	10622	14792	7417	7190
15	22164	6175	6175	12300	3645	3645
16	17276	7347	7004	10646	10283	9652[c]
17	4403	4252	3944	1904	1817	1685
18	3683	3550	3315	1683	1604	1498
19	4972	4756	4653	3319	3219	3150
20	9297	8922	8625	4715	4556	4405
21	14729	10356	9660	6958	3203	2988
22	2927	2819	2711	1798	1633	1570
23	2259	0	0	1366		
24	3397	0	0	2118		
25	4281	3228	3032	2048		
26	4165			2064		
27	3441			1694		
28	2581			1733		
29	804			806		
30	400			443		
May 1	216			317		
2	87			55		
3-13[d]	189			137		
Totals	132365	67813	64659	134198	67783	63573

[a]Total emergence to this date inclusive.

[b]1829 marked and released April 13.

[c]5392 marked and released April 17.

[d]Last observations May 13, emergence from any box less than 10/day.

Appendix Table 1 (cont'd)

TYPE FS

Date	Box 1			Box 4		
	No. emerged	No. marked	"Valid" marks	No. emerged	No. marked	"Valid" marks
Apr 9[a]	139			22		
10	39			6		
11	77			10		
12	1157			64		
13	4026			464		
14	2992			813		
15	6626	6626	6626	4007	1492	1492
16	10061	0	0	7889	5945	5759
17	2420	2228	2149	2844	2753	2656
18	2877	0	0	3530	0	0
19	3315	3194	3089	5403	5202	5031
20	6515	6284	5735	11935	5254	4795
21	16720	15434	14429[b]	23426	3292	2869
22	4972	4779	4757	5870	5670	5644
23	5227	4914	4914	7003	6659	6659
24	10293	5023	5023	9301	9052	9052
25	14529	2785	2785	13196	3334	3334
26	11119	3843	3793	9818	8812	8696
27	10338	8811	8811	9025	5759	5759
28	9857	4167	4065	9889	4735	4619
29	2909			3287		
30	2893			2649		
May 1	2052			1691		
2	982			694		
3-13[c]	665			571		
Totals	132968	68088	66176	133407	67959	66365

[a]Total emergence to this date inclusive.

[b]7868 marked and released on April 22.

[c]Last observations May 13, emergence from any box less than 10/day.

Appendix Table 1 (cont'd)

TYPE AM

Date	Box 2			Box 5		
	No. emerged	No. marked	"Valid" marks	No. emerged	No. marked	"Valid" marks
Apr 9[a]	21			19		
10	10			4		
11	12			3		
12	95			61		
13	384			495		
14	433			875		
15	2697			3794		
16	4709			6604	5382	5285
17	2103	1861	1840	1734	1607	1589
18	2705	2523	2523	1963	661	661
19	3607	3288	3288	4382	3605	3605
20	7032	5144	5083	10504	2227	2201
21	17661	0	0	22318	3059	3023
22	6158	5139	5086[b]	8594	8164	7918
23	8449	7937	7937	10914	7798	7798
24	11420	6452	6452	11736	9168	9168
25	16771	5203	5203	13866	0	0
26	13761	11484	11300	10033	8577	8440
27	12538	11990	11696	9288	8946	8727
28	15097	6965	6965	8912	8457	8457
29	3413			3488	379	379[c]
30	2731			2086		
May 1	1952			1178		
2	765			488		
3-13[d]	498			326		
Totals	135022	67986	67373	133655	68030	67251

[a]Total emergence to this date inclusive.

[b]3388 marked and released on April 23.

[c]Marked and released on April 28, but considered part of emergence for April 29.

[d]Last observations May 13, emergence from any box less than 10/day.

Appendix Table 2. Mean lengths, weights, and developmental indices (K_D) and their standard errors (SE) of samples of fry emerging daily from gravel boxes; 50 fish per sample. Means for replicate pairs are also given, weighted by daily emergences for boxes involved.

Date	Type and box no.		Length (mm)			Weight (mg)			K_D		
			$\bar{x}$	SE	Weighted mean for replicates	$\bar{x}$	SE	Weighted mean for replicates	$\bar{x}$	SE	Weighted mean for replicates
April 10	C	3	-	-	-	-	-	-	-	-	-
"	C	6	32.58	.185	-	276	4.9	-	1.994	.0060	-
"	FS	1	-	-	-	-	-	-	-	-	-
"	FS	4	-	-	-	-	-	-	-	-	-
"	AM	2	-	-	-	-	-	-	-	-	-
"	AM	5	-	-	-	-	-	-	-	-	-
April 11	C	3	-	-	-	-	-	-	-	-	-
"	C	6	33.01	.130	-	287	4.3	-	1.996	.0070	-
"	FS	1	-	-	-	-	-	-	-	-	-
"	FS	4	-	-	-	-	-	-	-	-	-
"	AM	2	-	-	-	-	-	-	-	-	-
"	AM	5	-	-	-	-	-	-	-	-	-
April 12	C	3	32.88	.134	32.83	284	3.0	288	1.997	.0068	2.010
"	C	6	32.82	.139	-	289	3.8	-	2.012	.0054	-
"	FS	1	32.30	.145	-	267	4.2	-	1.991	.0065	-
"	FS	4	-	-	-	-	-	-	-	-	-
"	AM	2	-	-	-	-	-	-	-	-	-
"	AM	5	-	-	-	-	-	-	-	-	-
April 15	C	3	33.08	.152	32.69	276	3.9	279	1.967	.0056	1.996
"	C	6	32.52	.156	-	280	4.1		2.008	.0071	-
"	FS	1	32.32	.162	-	265	3.8	-	1.986	.0057	-
"	FS	4	-	-	-	-	-	-	-	-	-
"	AM	2	-	-	-	-	-	-	-	-	-
"	AM	5	-	-	-	-	-	-	-	-	-
April 14	C	3	32.53	.125	32.51	281	3.9	276	2.013	.0084	2.002
"	C	6	32.50	.102	-	273	2.8	-	1.994	.0047	-
"	FS	1	32.42	.157	32.51	274	4.2	275	2.001	.0062	1.998
"	FS	4	32.84	.151	-	280	3.5	-	1.991	.0056	-
"	AM	2	32.57	.141	32.29	273	4.3	266	1.988	.0052	1.987
"	AM	5	32.15	.231	-	263	5.7	-	1.986	.0059	-

Appendix Table 2 (cont'd)

Date	Type and box no.	Length (mm) $\bar{x}$	Length (mm) SE	Length (mm) Weighted mean for replicates	Weight (mg) $\bar{x}$	Weight (mg) SE	Weight (mg) Weighted mean for replicates	K_D $\bar{x}$	K_D SE	K_D Weighted mean for replicates
April 15	C 3	32.72	.113	32.64	288	3.4	284	2.016	.0052	2.011
"	C 6	32.49	.136		276	3.9		2.002	.0062	
"	FS 1	32.56	.146	32.63	278	3.9	281	2.002	.0050	2.006
"	FS 4	32.75	.120		287	4.0		2.012	.0058	
"	AM 2	32.90	.129	32.78	282	4.0	277	1.989	.0066	1.986
"	AM 5	32.68	.187		274	5.1		1.983	.0063	
April 16	C 3	32.60	.154	32.62	280	4.1	279	2.003	.0050	2.001
"	C 6	32.64	.116		278	3.2		1.997	.0062	
"	FS 1	32.65	.127	32.75	274	4.4	278	1.985	.0071	1.989
"	FS 4	32.87	.141		282	3.6		1.994	.0057	
"	AM 2	32.76	.113	32.94	279	3.2	282	1.992	.0057	1.989
"	AM 5	33.07	.138		285	3.8		1.987	.0050	
April 17	C 3	32.46	.138	32.46	275	3.5	276	2.002	.0051	2.005
"	C 6	32.46	.115		279	3.3		2.011	.0046	
"	FS 1	32.71	.156	32.67	281	4.5	276	2.000	.0057	1.991
"	FS 4	32.64	.140		272	3.5		1.982	.0057	
"	AM 2	32.84	.154	32.94	282	4.1	284	1.995	.0053	1.994
"	AM 5	33.06	.156		287	4.3		1.992	.0049	
April 18	C 3	32.75	.134	32.69	283	3.2	282	2.003	.0054	2.004
"	C 6	32.56	.134		279	3.4		2.006	.0044	
"	FS 1	32.76	.142	32.75	276	3.4	276	1.985	.0054	1.987
"	FS 4	32.74	.149		277	3.5		1.989	.0049	
"	AM 2	33.05	.137	32.96	285	3.8	282	1.989	.0050	1.987
"	AM 5	32.83	.177		278	4.8		1.985	.0055	
April 19	C 3	32.80	.144	32.65	275	3.8	275	1.981	.0050	1.991
"	C 6	32.41	.156		276	4.3		2.005	.0060	
"	FS 1	32.78	.142	32.92	277	4.1	279	1.985	.0054	1.982
"	FS 4	33.01	.128		280	3.8		1.980	.0066	
"	AM 2	33.37	.137	33.21	289	3.5	289	1.980	.0053	1.988
"	AM 5	33.08	.172		288	4.2		1.995	.0048	

Appendix Table 2 (cont'd)

Date	Type and box no.		Length (mm) $\bar{x}$	Length (mm) SE	Length (mm) Weighted mean for replicates	Weight (mg) $\bar{x}$	Weight (mg) SE	Weight (mg) Weighted mean for replicates	K_D $\bar{x}$	K_D SE	K_D Weighted mean for replicates
April 20	C	3	32.62	.146	32.64	274	4.2	277	1.988	.0064	1.995
"	C	6	32.68	.104		284	2.8		2.010	.0056	
"	FS	1	33.14	.135	33.08	283	3.7	279	1.979	.0050	1.973
"	FS	4	33.04	.160		277	4.2		1.970	.0055	
"	AM	2	33.19	.138	33.07	287	3.6	284	1.987	.0055	1.986
"	AM	5	32.98	.124		282	3.7		1.985	.0051	
April 21	C	3	32.52	.143	32.43	272	4.1	271	1.990	.0065	1.992
"	C	6	32.23	.128		268	3.7		1.998	.0071	
"	FS	1	32.65	.168	32.76	278	4.2	277	1.996	.0051	1.988
"	FS	4	32.85	.148		277	3.9		1.981	.0048	
"	AM	2	33.15	.108	33.03	280	3.3	276	1.972	.0051	1.970
"	AM	5	32.94	.142		273	3.7		1.968	.0072	
April 22	C	3	32.64	.148	32.57	267	3.6	268	1.972	.0067	1.978
"	C	6	32.47	.148		269	3.8		1.987	.0055	
"	FS	1	32.70	.145	32.74	268	4.1	269	1.968	.0056	1.969
"	FS	4	32.78	.152		271	4.4		1.970	.0052	
"	AM	2	33.04	.145	33.09	277	3.6	283	1.971	.0051	1.981
"	AM	5	33.13	.166		287	4.2		1.989	.0053	
April 23	C	3	32.52	.142	32.61	268	3.7	273	1.980	.0066	1.989
"	C	6	32.74	.128		283	3.3		2.003	.0054	
"	FS	1	32.86	.147	32.87	285	4.2	280	1.999	.0043	1.989
"	FS	4	32.87	.156		277	4.1		1.981	.0055	
"	AM	2	32.95	.153	32.99	277	4.7	284	1.974	.0055	1.989
"	AM	5	33.02	.139		289	3.5		2.001	.0053	
April 24	C	3	32.13	.164	32.30	271	4.2	272	2.012	.0065	2.004
"	C	6	32.57	.142		273	3.6		1.991	.0059	
"	FS	1	32.68	.147	32.73	275	4.1	275	1.986	.0049	1.986
"	FS	4	32.78	.131		276	3.5		1.985	.0048	
"	AM	2	33.20	.169	33.07	291	4.5	283	1.994	.0071	1.984
"	AM	5	32.94	.130		276	3.4		1.973	.0053	

Appendix Table 2 (cont'd)

Date	Type and box no.	Length (mm) $\bar{x}$	Length (mm) SE	Length (mm) Weighted mean for replicates	Weight (mg) $\bar{x}$	Weight (mg) SE	Weight (mg) Weighted mean for replicates	K_D $\bar{x}$	K_D SE	K_D Weighted mean for replicates
April 25	C 3	32.47	.116	32.50	266	3.1	263	1.979	.0056	1.971
"	C 6	32.56	.126		258	3.5		1.953	.0047	
"	FS 1	32.62	.144	32.72	266	3.9	269	1.968	.0064	1.970
"	FS 4	32.82	.111		272	3.2		1.972	.0054	
"	AM 2	32.70	.140	32.89	273	3.3	272	1.983	.0050	1.969
"	AM 5	33.13	.122		271	3.5		1.952	.0053	
April 26	C 3	32.60	.129	32.52	261	3.2	261	1.959	.0055	1.963
"	C 6	32.35	.145		259	3.4		1.969	.0064	
"	FS 1	32.74	.134	32.84	268	3.9	270	1.968	.0055	1.966
"	FS 4	32.96	.130		272	3.5		1.964	.0052	
"	AM 2	33.01	.150	32.98	273	4.1	271	1.962	.0056	1.960
"	AM 5	32.94	.139		269	3.6		1.957	.0050	
April 27	C 3	32.46	.165	32.43	260	4.3	261	1.964	.0064	1.969
"	C 6	32.38	.127		264	3.5		1.978	.0054	
"	FS 1	32.50	.135	32.63	264	3.6	269	1.972	.0052	1.976
"	FS 4	32.79	.137		275	3.9		1.981	.0054	
"	AM 2	32.59	.121	32.70	274	3.4	273	1.992	.0060	1.981
"	AM 5	32.85	.142		271	4.1		1.967	.0057	
April 28	C 3	32.29	.161	32.31	261	3.8	262	1.977	.0063	1.978
"	C 6	32.36	.142		264	3.5		1.981	.0061	
"	FS 1	32.34	.126	32.53	259	3.5	262	1.968	.0069	1.964
"	FS 4	32.71	.142		265	3.9		1.960	.0061	
"	AM 2	32.76	.181	32.89	273	4.3	275	1.977	.0060	1.974
"	AM 5	33.09	.118		278	3.7		1.970	.0051	
April 29	C 3	32.01	.161	32.19	252	3.6	254	1.973	.0075	1.967
"	C 6	32.36	.150		256	3.2		1.961	.0062	
"	FS 1	32.52	.172	32.65	251	4.2	256	1.958	.0059	1.952
"	FS 4	32.77	.169		261	4.0		1.947	.0069	
"	AM 2	32.80	.146	32.92	269	4.1	268	1.965	.0054	1.957
"	AM 5	33.04	.140		268	3.7		1.949	.0062	

Appendix Table 2 (cont'd)

Date	Type and box no.	Length (mm)			Weight (mg)			K_D		
		$\bar{x}$	SE	Weighted mean for replicates	$\bar{x}$	SE	Weighted mean for replicates	$\bar{x}$	SE	Weighted mean for replicates
April 30	C 3	31.88	.221	32.00	249	4.4	252	1.972	.0081	1.972
"	C 6	32.10	.139		254	3.5		1.971	.0068	
"	FS 1	32.88	.135	32.93	260	3.8	263	1.939	.0050	1.944
"	FS 4	32.98	.137		267	3.6		1.950	.0055	
"	AM 2	33.03	.175	32.94	268	4.0	267	1.949	.0063	1.952
"	AM 5	32.82	.162		266	3.6		1.957	.0077	
May 1	C 3	32.22	.171	32.20	254	3.7	253	1.965	.0065	1.962
"	C 6	32.18	.245		252	4.3		1.961	.0082	
"	FS 1	32.93	.147	32.92	261	3.1	261	1.939	.0064	1.940
"	FS 4	32.92	.198		262	4.1		1.940	.0056	
"	AM 2	33.02	.218	33.01	266	4.4	267	1.944	.0050	1.948
"	AM 5	33.00	.162		270	3.7		1.956	.0058	
May 2	C 3	-	-	-	-	-	-	-	-	-
"	C 6	-	-		-	-		-	-	
"	FS 1	32.75	.142	32.71	261	3.2	263	1.949	.0062	1.958
"	FS 4	32.65	.152		267	3.5		1.970	.0057	
"	AM 2	32.97	.167	33.01	266	3.4	268	1.950	.0071	1.952
"	AM 5	33.08	.131		271	2.7		1.956	.0058	
May 3	C 3	-			-	-	-	-	-	-
"	C 6	-			-	-		-	-	
"	FS 1	32.78	.155	32.54	259	3.5	259	1.943	.0073	1.959
"	FS 4	32.26	.173		260	3.5		1.977	.0100	
"	AM 2	32.48	.189	-	265	3.2	-	1.978	.0091	-
"	AM 5	-	-		-	-		-		

Appendix Table 3. Mean lengths, weights, and developmental indices (K_D) and their standard errors and confidence limits for samples of fry emerging from gravel boxes.

Character	Type and box no.		Sample type	n	df	$\bar{x}$	SE	Confidence limits for sample mean	
								Lower	Upper
Length (mm)	C	3	Unwt'd	1000	999	32.51	.034	32.44	32.58
"	C	3	Wt'd	1000	308	32.64	.041	32.56	32.72
"	C	3	Sub S	200	199	32.64	.075	32.49	32.79
"	C	6	Unwt'd	1100	1099	32.50	.031	32.44	32.56
"	C	6	Wt'd	1100	141	32.58	.052	32.48	32.68
"	C	6	Sub S	200	199	32.54	.071	32.40	32.68
"	FS	1	Unwt'd	1100	1099	32.66	.031	32.60	32.72
"	FS	1	Wt'd	1100	333	32.65	.039	32.57	32.72
"	FS	1	Sub S	200	199	32.62	.082	32.46	32.79
"	FS	4	Unwt'd	1000	999	32.80	.033	32.74	32.87
"	FS	4	Wt'd	1000	237	32.85	.041	32.77	32.93
"	FS	4	Sub S	200	199	32.81	.070	32.67	32.95
"	AM	2	Unwt'd	1000	999	32.92	.035	32.85	32.99
"	AM	2	Wt'd	1000	352	32.93	.042	32.85	33.02
"	AM	2	Sub S	200	199	32.99	.075	32.84	33.14
"	AM	5	Unwt'd	950	949	32.94	.036	32.87	33.01
"	AM	5	Wt'd	950	294	32.99	.040	32.91	33.07
"	AM	5	Sub S	200	199	33.04	.066	32.91	33.17
Weight (mg)	C	3	Unwt'd	1000	999	270.0	0.90	268.2	271.7
"	C	3	Wt'd	1000	295	276.7	1.14	274.4	278.9
"	C	3	Sub S	200	199	276.9	2.00	273.0	280.9
"	C	6	Unwt'd	1100	1099	271.6	0.84	270.0	273.3
"	C	6	Wt'd	1100	145	278.4	1.40	275.6	281.1
"	C	6	Sub S	200	199	277.8	1.97	273.9	281.6
"	FS	1	Unwt'd	1100	1099	269.9	0.86	268.2	271.6
"	FS	1	Wt'd	1100	383	271.0	1.05	269.0	273.1
"	FS	1	Sub S	200	199	270.9	2.16	266.6	275.1
"	FS	4	Unwt'd	1000	999	272.8	0.87	271.1	274.6
"	FS	4	Wt'd	1000	252	274.5	1.10	272.3	276.6
"	FS	4	Sub S	200	199	273.9	1.95	270.1	277.8
"	AM	2	Unwt'd	1000	1099	276.4	0.89	274.7	278.2
"	AM	2	Wt'd	1000	382	277.6	1.11	295.4	279.8
"	AM	2	Sub S	200	199	278.9	1.89	275.2	282.7
"	AM	5	Unwt'd	950	949	276.0	0.94	274.2	277.9
"	AM	5	Wt'd	950	314	276.9	1.08	274.7	279.0
"	AM	5	Sub S	200	199	276.7	1.78	273.2	280.2
K_D	C	3	Unwt'd	1000	999	1.986	.0015	1.983	1.989
"	C	3	Wt'd	1000	321	1.994	.0018	1.991	1.997
"	C	3	Sub S	200	199	1.995	.0036	1.998	2.002
"	C	6	Unwt'd	1100	1099	1.991	.0014	1.988	1.993
"	C	6	Wt'd	1100	145	2.002	.0023	1.998	2.007
"	C	6	Sub S	200	199	2.003	.0032	1.997	2.009
"	FS	1	Unwt'd	1100	1099	1.976	.0014	1.974	1.979
"	FS	1	Wt'd	1100	388	1.980	.0015	1.978	1.983
"	FS	1	Sub S	200	199	1.980	.0032	1.974	1.987

Appendix Table 3 (cont'd)

Character	Type and box no.		Sample type	n	df	$\bar{x}$	SE	Confidence limits for sample mean	
								Lower	Upper
K_D	FS	4	Unwt'd	1000	999	1.975	.0014	1.972	1.978
"	FS	4	Wt'd	1000	330	1.976	.0015	1.973	1.979
"	FS	4	Sub S	200	199	1.977	.0028	1.972	1.983
"	AM	2	Unwt'd	1000	999	1.977	.0014	1.974	1.980
"	AM	2	Wt'd	1000	385	1.979	.0016	1.976	1.982
"	AM	2	Sub S	200	199	1.979	.0028	1.973	1.984
"	AM	5	Unwt'd	950	949	1.975	.0014	1.972	1.977
"	AM	5	Wt'd	950	194	1.974	.0017	1.971	1.977
"	AM	5	Sub S	200	199	1.971	.0029	1.966	1.977

Sample type: Unwt'd: values from daily samples of 50 fry pooled and considered as single sample.

Wt'd: estimate from daily samples of 50 weighted by numbers emerging daily.

Sub S: values from randomly selected sub-samples of 200.

n: number of fish sampled.

df: degrees of freedom [for the weighted estimates, the effective degrees of freedom calculated according to Cochran (1963)].

$\bar{x}$: sample mean.

SE: standard error of sample mean.

Confidence limits are for α =.95.